1 **Supplemantary Information for "*In vivo* KRAS G12D/V Degradation Mediated by**

2 **CANDDY Using a Modified Proteasome Inhibitor"**

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8 **General.** Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL

9 JNM-ECS-400 spectrometer operating at 400 MHz for ^{1}H-NMR. Chemical shifts were

10 reported in the scale relative to solvent described respectively as an internal reference.

11 High resolution fast-atom bombardment mass spectrometry (HR-MS FAB) was

12 performed on a JEOL JMS-700. Column chromatography was performed with silica gel

13 60N (spherical, neutral) purchased from Kanto Chemical Co., Inc. and with NH silica

14 gel (NH-DM1020) purchased from Fuji Silysia Chemical Ltd. Reversed phase liquid

15 chromatography was performed with high performance liquid chromatography (HPLC)

16 (Shimadzu Co., Ltd). HPLC experiment for the term of 'The synthesis of TUS-007' was

17 performed with a TSK-Gel ODS-80TM (Tosoh Corp.) (4.6 x 150 mm) with a gradient

18 of 5-95% acetonitrile in water with 0.1% trifluoroacetic acid (0 min 5%, 2 min 5%, 15

19 min 95%, 20 min 95%, 1 mL/min, 40°C). HPLC experiment for the term of 'the

20 synthesis of MDM2-CANDDY' was performed with a Inertsil ODS-4 (GL Science) (4.6

21 x 250 mm) with isocratic condition (MeCN/water/TFA=750/250/1, 1.0 mL/min, 40°C),

22 unless otherwise noted.

23 Glycil-L-leucine was purchased from Combi-Blocks Inc. *N*-Boc-L-isoleucine was

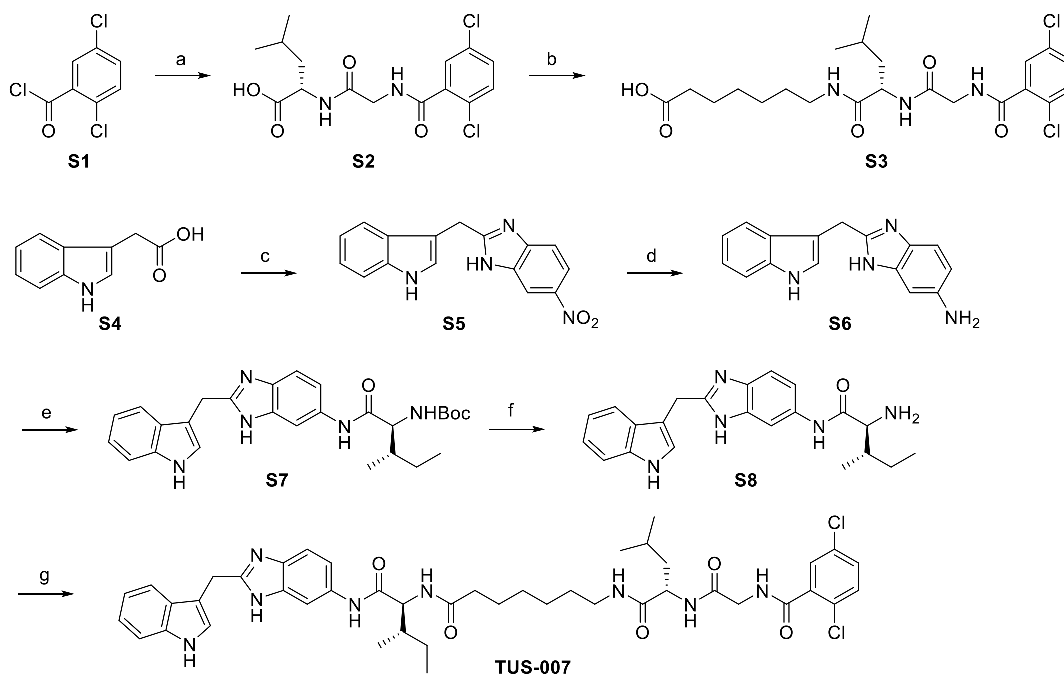
24 purchased from Watanabe Chemical Industry.

25

26 **The synthesis of TUS-007.**

27 TUS-007 was synthesized as shown below. Some of the compounds were synthesized

28 by NARD Institute. Ltd. (Kobe, Japan).



29 Reagent and conditions: (a) Glycyl-L-luecine, 1N NaOHaq.,THF 0°C, 88%; (b) (i) HOSu, EDC-HCl, CH_2Cl_2, 0°C to rt, (ii) 7-Amino-heptanoic acid, Triethylamine, DMF, 0°C to rt, 72%; (c) (i) CDI, THF, 0°C to rt; 4-Nitro-1,2-phenylenediamine, 47°C, (ii) AcOH, reflux, 88%; (d) H_2, Pd/C, MeOH, rt, 62%; (e) *N*-Boc-L-Isoleucine, COMU, DIPEA, MeCN, 0°C to rt, *quant.*; (f) TFA, CH_2Cl_2, rt, 76%; (g) **S3**, HATU, DIPEA, DMF, 0°C to rt, 53%.

30 To a solution of glycyl-L-leucine (125 g, 664 mmol) in 1N NaOH*aq*. (1.3 L)

31 with tetrahydrofuran (THF: 550 mL), 2,5-dichlorobenzoyl chloride (127 g, 604 mmol,

32 **S1**) in THF (250 mL) was added dropwise at 0°C. After stirring for 2 h at the same

33 temperature, the resulting solution was acidified (ca. pH2) with 2N HCl*aq*. and

34 extracted with $CHCl_3$ (total 3 L). The combined organic layer was washed with brine,

35 dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was suspended in

36 diisopropyl ether (IPE: 1.2 L) and stirred for 30 min at 50°C. The resulting suspension

37 was cooled to room temperature, and a precipitate was filtered, washed with IPE (500

38 mL), and thus **S2** (194 g, 88 % yield, white solid) was obtained. **S2**: ^{1}H-NMR (400 MHz,

39 DMSO-d_6) δ 8.77 (t, J = 6.0 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.54 (s, 3H), 4.24-4.30

40 (m, 1H), 3.84-3.95 (m, 2H), 1.60-1.70 (m, 1H), 1.48-1.59 (m, 2H), 0.90 (d, J = 6.4 Hz,

41 3H), 0.86 (d, J = 6.4 Hz, 3H). HPLC: t_R = 11.82 min, purity; 94.0% (λ = 254 nm).

42 (i) To a solution of **S2** (194g, 536 mmol) in CH_2Cl_2 (1.6 L), *N*-hydroxysuccinimide

43 (HOSu: 2.5 g, 804 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

44 hydrochloride (EDC-HCl: 54 g, 804 mmol) were added at 0°C under Ar atmosphere.

45 After stirring for 3 h at room temperature, the resulting solution was washed with water

46 (1 L) three times, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. (ii) The

47 residue (309 g) was dissolved in DMF (1.5 L) at Ar atmosphere, and 7-aminoheptanoic

48 acid (77.8 g, 536 mmol) and triethylamine (178 mL) were added to the solution at 0°C.

49 After stirring overnight at room temperature, 1N HCl*aq*. (1.5 L), water (1 L) and AcOEt

50 (3 L) were added and extraction was performed using AcOEt (1.5 L). The combined

51 organic layer was dried over Na_2SO_4, filtered, concentrated *in vacuo* and azeotroped

52 with toluene. The residue (394 g) was purified by silica gel column chromatography

53 (4.5 kg, elution with 0-5 % MeOH/$CHCl_3$) and thus **S3** (190 g, 72 % yield, white solid)

54 was obtained. **S3**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1H), 8.78 (t, *J* = 6.0 Hz,

55 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.92 (t, *J* = 5.5 Hz, 1H), 7.53 (s, 3H), 4.26-4.31 (m, 1H),

56 3.82-3.92 (m, 2H), 2.94-3.09 (m, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.35-1.60 (m, 7H),

57 1.18-1.27 (m, 4H), 0.87 (d, *J* = 6.4 Hz, 3H), 0.84 (d, *J* = 6.4 Hz, 3H).

58 (i) To a solution of indol-3-ylacetic acid (100 g, 571 mmol, **S4**) in THF (200 mL),

59 1,1'-Carbonyldiimidazole (CDI: 109 g, 674 mmol) was added in portions over 15 min at

60 0°C under Ar atmosphere. After stirring for 30 min at room temperature,

61 4-nitro-1,2-phenylenediamine (96.2 g, 628 mmol) was added, and stirred for 30 min at

62 same temperature. The resulting solution was warmed to 47°C and concentrated *in*

63 *vacuo* after stirring overnight. (ii) The residue was dissolved in AcOH (2 L) with

64 ice-cooling and heated to reflux for 2 h. The resulting solution was concentrated *in*

65 *vacuo* and azeotroped with toluene. The residue was dissolved in AcOEt (1 L), and

66 insoluble portion was filtered on a Celite-pad, then washed with AcOEt (0.8 L). The

67 filtrate was washed with water (1.5 L) and brine (1 L), dried over Na_2SO_4, and

68 concentrated *in vacuo*. The residue (298 g) was purified by silica gel column

69 chromatography (2 kg, elution with 0-30 % AcOEt/$CHCl_3$) and thus **S5** (146 g, 88 %

70 yield, brown amorphous) was obtained. **S5**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.03 (s,

71 1H), 9.67 (s, 1H), 8.02-8.42 (m, 2H), 7.28-7.70 (m, 4H), 6.93-7.10 (m, 2H), 4.37 (s,

72 2H).

73 To a solution of **S5** (146 g, 500 mmol) in MeOH (1 L), Pd/C (20.0 g) was added and

74 stirred overnight at room temperature under H_2 atmosphere. The resulting solution was

75 filtered on a Celite-pad, washed with MeOH (2 L), and concentrated *in vacuo*. The

76 residue was purified by silica gel column chromatography (3.0 kg, elution with 5-20 %

77 MeOH/$CHCl_3$) and thus **S6** (85.4 g, 62 % yield, black solid) was obtained. **S6**: ^{1}H-NMR

78 (400 MHz, DMSO-d_6) δ 7.45-7.47 (m, 1H), 7.33-7.35 (m, 1H), 7.24-7.25 (m, 1H),

79 7.11-7.13 (m, 1H), 7.03-7.07 (m, 1H), 6.91-6.94 (m, 1H), 6.56-6.57 (m, 1H), 6.42-6.44

80 (m, 2H), 4.16 (s, 2H), 3.35 (br s, 2H). HPLC: t_R = 9.08 min, purity; 97.9% (λ = 254 nm).

81 HPLC: t_R = 12.26 min, purity; 87.2% (λ = 254 nm).

82 To a solution of **S6** (81.9 g, 351 mmol) in MeCN (1.2 L), *N*-ethyldiisopropylamine

83 (DIPEA: 119 mL) and (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-

84 morpholinocarbenium Hexafluorophosphate (COMU: 58 g, 368 mmol) were added at

85 0°C under Ar atmosphere, and stirred for 1 h at room temperature. After cooling to 0°C,

86 *N*-Boc-L-isoleucine (89.4 g, 341 mmol) was added and stirred overnight at room

87 temperature, and then concentrated *in vacuo*. The residue was diluted with $CHCl_3$ (2 L)

88 and washed with water (1.5 L) twice, concentrated *in vacuo*, and thus **S7** (330 g, *quant*.,

89 black oil) was obtained. This compound was used in the next step without further

90 purification.

91 To a solution of **S7** (330 g) in CH_2Cl_2 (1.6 L), trifluoroacetic acid (TFA: 800 mL) was

92 added at room temperature under Ar atmosphere and stirred for 2 h at the same

93 temperature. The resulting solution was concentrated *in vacuo*, and then dissolved in

94 AcOEt (1 L), washed with *sat*. $NaHCO_3$*aq*. (600 mL) twice, dried over Na_2SO_4, filtered,

95 and concentrated *in vacuo*. The residue (315 g) was purified by NH-silica gel column

96 chromatography (2.3 kg, elution with 0-5 % MeOH/$CHCl_3$), and thus **S8** (97.5 g, 76 %

97 yield, yellow solid) was obtained. **S8**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H),

98 10.94 (s, 1H), 9.72 (s, 1H), 7.95-7.13 (m, 6H), 7.07-7.03 (m, 1H), 6.95-6.91 (m, 1H),

99 4.23 (s, 2H), 3.18-3.11 (m, 1H), 1.67-1.48 (m, 2H), 1.21-1.06 (m, 1H), 0.95-0.81 (m,

100 6H). HPLC: t_R = 9.80 min, purity; 87.8% (λ = 254 nm).

101 To a solution of **S3** (139 g, 285 mmol) in *N*,*N*-dimethylformamide (DMF: 1250 mL),

102 DIPEA (77.1 mL) and

103 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide

104 hexafluorophosphate (HATU: 109 g, 259 mmol) were added at 0°C under Ar

105 atmosphere. After stirring for 30 min at the same temperature, **S8** (97.4 g, 259 mmol)

106 was added at 0°C and stirred overnight at room temperature. The resulting solution was

107 diluted with AcOEt (2.5 L) and water (1.5 L) and extracted with AcOEt (1.5 L). The

108 combined organic layer was concentrated *in vacuo* and azeotroped with toluene. The

109 residue (448 g) was purified by NH-silica gel (6.0 kg, elution with 0-4 %

110 MeOH/$CHCl_3$) and concentrated *in vacuo*. After dissolving in MeOH (500 mL),

111 insoluble was filtered off and the filtrate was concentrated *in vacuo*. The residue was

112 suspended in Me_2CO (1.5 L), stirred, and cooled to room temperature. Subsequently, a

113 precipitate was collected, and thus **TUS-007** (117 g, 53 % yield, yellow solid) was

114 obtained.**TUS-007**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.93-11.97 (m, 1H), 10.92 (s,

115 1H), 9.96 (d, *J* = 22.4 Hz, 1H), 8.78 (t, *J* = 5.7 Hz, 1H), 7.88-7.97 (m, 4H), 7.52 (s, 3H),

116 7.34 (d, *J* = 8.2 Hz, 1H), 7.14-7.44 (m, 4H), 7.02-7.06 (m, 1H), 6.89-6.93 (m, 1H),

117 4.22-4.38 (m, 4H), 3.81-3.92 (m, 2H), 2.94-3.08 (m, 2H), 2.07-2.21 (m, 2H), 1.75-1.82

118 (m, 1H), 1.36-1.59 (m, 8H), 1.08-1.28 (m, 5H), 0.87 (d, *J* = 6.4 Hz, 3H), 0.74-0.88 (m,

119 6H), 0.83 (d, *J* = 6.4 Hz, 3H). HR-MS (FAB) *m/z*: 845.3674 (Calcd for $C_{44}H_{55}Cl_2N_8O_5$

120 $[M+H]^+$ 845.3672). HPLC: t_R = 13.09 min, purity; 96.7% (λ = 254 nm).

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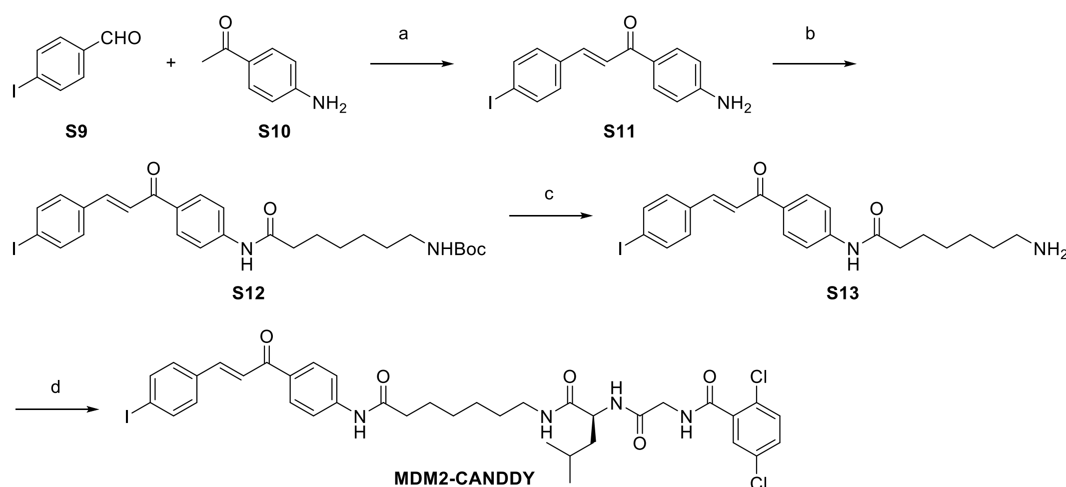
123 **The synthesis of MDM2-CANDDY.**

124 MDM2-CANDDY was synthesized as shown below. Some of the compounds were
125 synthesized by NARD Institute. Ltd. (Kobe, Japan).



126 Reagent and conditions: (a) 40% KOHaq., EtOH, 0°C to rt, 60%; (b) 7-{(*tert*-Butoxycarbonyl)amino}heptanoic acid, HATU, DIPEA, DMAP, DMA/CH_2Cl_2, 0°C to rt, 70%; (c) TFA, CH_2Cl_2, 0°C, 99%; (d) CANDDY-MLN, HATU, DIPEA, DMF/CH_2Cl_2, 0°C to rt, 65%.

127 To a solution of 4-Iodobenzaldehyde (7.00 g, 30.1 mmol, **S9**) and
128 4′-Aminoacetophenone (4.08 g, 30.1 mmol, **S10**) in EtOH (90 mL), 40% KOH*aq*. (60
129 mL) was added dropwise over 1 h at 0°C and stirred for 1 h under Ar atmosphere. After
130 stirring for 6 h at room temperature, the resulting solution was cooled to 12°C. A
131 precipitate was collected by filtration and washed with EtOH-water (1:2, 80 mL). The
132 collected solid was dissolved in $CHCl_3$ (800 mL) and the insoluble content was filtered
133 and removed. The filtrate was washed with water twice, dried over Na_2SO_4, filtered,

134 concentrated *in vacuo*, and thus **S11** (6.27 g, 60% yield, yellow solid) was obtained.

135 This compound was used without further purification. **S11**: ^{1}H-NMR (400 MHz,

136 DMSO-d_6) δ 6.91-6.86 (m, 3H), 6.78 (d, *J* = 8.2 Hz, 2H), 6.62 (d, *J* = 8.2 Hz, 2H), 6.52

137 (d, *J* = 15.2 Hz, 1H), 5.86 (d, *J* = 8.8 Hz, 2H), 5.16 (s, 2H).

138 To a solution of **S11** (6.16 g, 17.7 mmol), 7-{(*tert*-Butoxycarbonyl)amino}heptanoic

139 acid (4.35 g, 17.7 mmol), and DIPEA (4.62 mL, 26.5 mmol) in CH_2Cl_2-*N*,*N*-dimethyl-

140 acetamide (DMA: 10:1, 170 mL), HATU (10.2 g, 26.8 mmol) was added at 0°C under

141 Ar atmosphere. After stirring for 1 h at the same temperature, the solution was warmed

142 to room temperature and stirred for 4 h, and then 4-dimethylaminopyridine (DMAP:

143 250 mg, 2.04 mmol) was added, and stirred further 17 h at ambient temperature. After

144 stirring for 48 h at 30°C, the resulting solution was diluted with CH_2Cl_2 (1.2 L) and

145 washed with water (0.6 L), 1N HClaq. (0.6 L) twice, sat. $NaHCO_3$aq. (0.6 L) and brine

146 (0.6 L). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated

147 in vacuo. The residue was purified by silica gel column chromatography (elution with

148 10-50% AcOEt/heptane). Next, the resulting rough solid was suspended in AcOEt (150

149 mL) at 50°C for 1 h and IPE (300 mL) was added, and the mixture was cooled on

150 ice-bath. A precipitate was collected, and thus **S12** (7.14 g, 70% yield, pale yellow

151 solid) was obtained. **S12**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.8 Hz, 2H), 7.97

152 (s, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.75-7.72 (m, 3H), 7.72 (d, *J* = 15.6 Hz, 1H), 7.54 (d,

153 *J* = 15.6 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 2H), 3.13 (m, 2H), 2.39 (t, *J* = 6.8 Hz, 2H),

154 1.79-1.72 (m, 2H), 1.49-1.34 (m, 6H), 1.46 (s, 9H). HPLC: t_R = 8.74 min, purity; 97.1%

155 (λ = 254 nm).

156 To a solution of **S12** (2.50 g, 4.34 mmol) in CH_2Cl_2 (125 mL), TFA (63 mL)

157 was added at 0°C under Ar atmosphere. After stirring 1.5 h at the same temperature, the

158 resulting solution was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2,

159 poured into sat. $NaHCO_3$aq./water (2:1, 300 mL) and stirred for 1 h. The precipitate was

160 collected by filtration, washed with water (20 mL) twice, dried *in vacuo*, and thus **S13**

161 (2.05g, 99% yield, pale orange solid) was obtained. **S13**: ^{1}H-NMR (400 MHz,

162 CF_3CO_2D) δ 8.13 (d, *J* = 8.7 Hz, 2H), 7.92 (d, *J* = 15.5 Hz, 1H), 7.86 (d, *J* = 8.2 Hz,

163 2H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.66 (d, *J* = 15.5 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 2H), 6.80

164 (s, 1H), 3.33-3.31 (m, 2H), 2.71 (t, *J* = 7.8 Hz, 2H), 1.92-1.91 (m, 4H), 1.61-1.58 (m,

165 4H). HPLC (MeCN/water/TFA=500/500/1): t_R = 9.85 min, purity; 95.7% (λ = 254 nm).

166 To a solution of **CANDDY_MLN** (MLN2238 derived CANDDY tag: 0.72 g, 2.0 mmol,

167 synthesized by WO2018092723) in DMF/CH_2Cl_2 (1:1, 20 mL), DIPEA (0.32 mL, 1.9

168 mmol) and HATU (0.76 g, 2.0 mmol) were added at 3°C. After stirring at same

169 temperature for 30 min, **S13** (0.90g, 1.9 mmol) was added and warmed to room

170 temperature, stirred for further 3 h at room temperature. The resulting solution was

171 evaporated, diluted with water (200 mL), and stirred. A precipitate was collected by

172 filtration, dried *in vacuo*, and thus **MDM2-CANDDY** (1.0 g, 65% yield, white solid)

173 was obtained. **MDM2-CANDDY**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H),

174 8.78 (dd, *J* = 5.9, 5.9 Hz, 1H), 8.12 (d, *J* = 8.9 Hz, 2H), 7.96 (d, *J* = 15.8 Hz, 1H),

175 7.91-7.98 (m, 2H), 7.82 (d, *J* = 8.5 Hz, 2H), 7.76 (d, *J* = 8.9 Hz, 2H), 7.67 (d, *J* = 8.5 Hz,

176 2H), 7.64 (d, *J* = 15.8 Hz, 1H), 7.52 (s, 3H), 4.25-4.31 (m, 1H), 3.81-3.91 (m, 2H),

177 2.97-3.07 (m, 2H), 2.33 (t, *J* = 7.2 Hz, 2H), 1.53-1.59 (m, 3H), 1.38-1.46 (m, 4H),

178 1.25-1.31 (m, 4H), 0.87 (d, *J* = 6.5 Hz, 3H), 0.84 (d, *J* = 6.5 Hz, 3H). HR-MS (FAB)

179 *m*/*z*: 819.1577 (Calcd for $C_{37}H_{42}Cl_2IN_4O_5$ $[M+H]^+$ 819.1577). HPLC: t_R = 7.99 min,

180 purity; 95.7% (λ = 254 nm).

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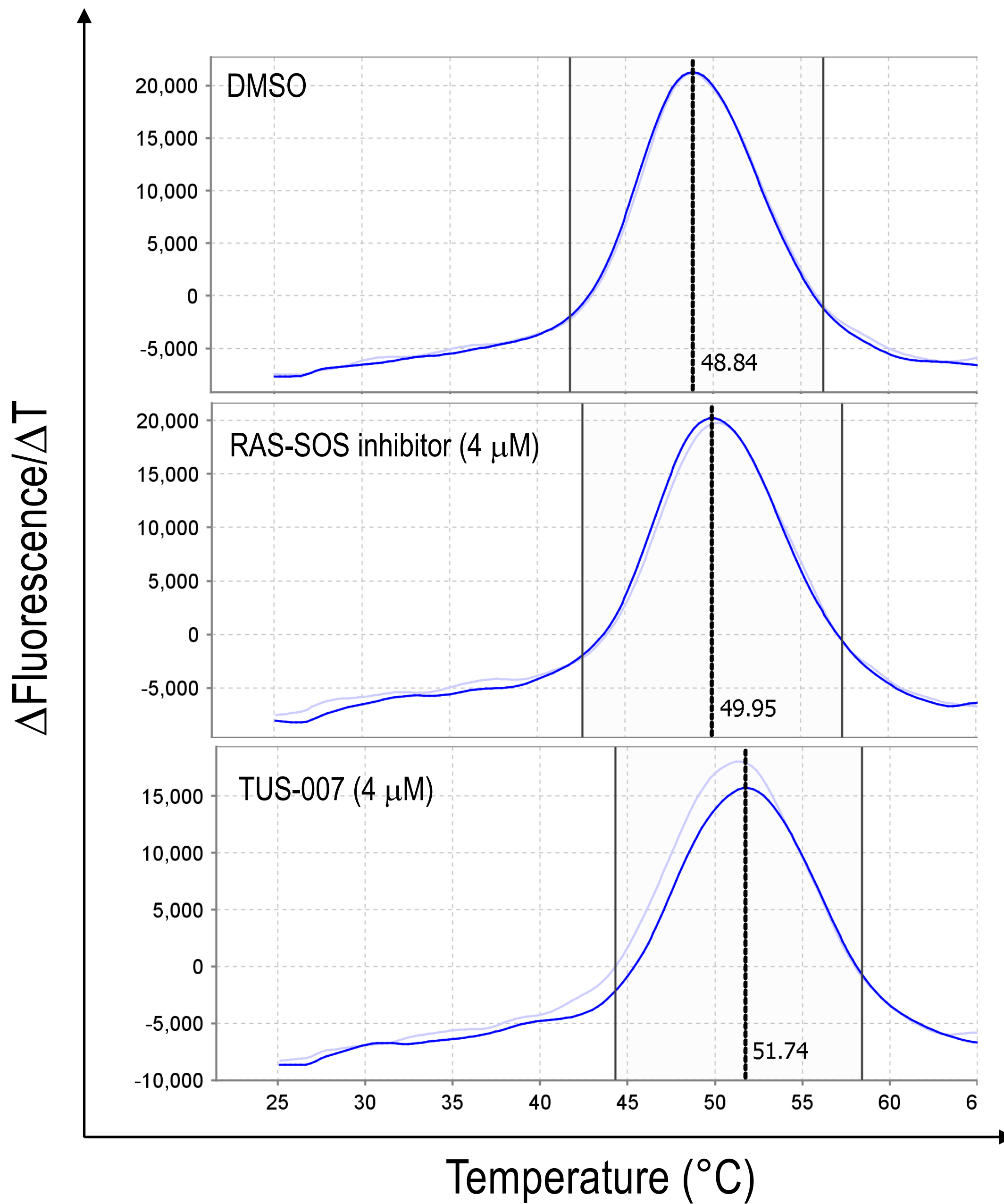
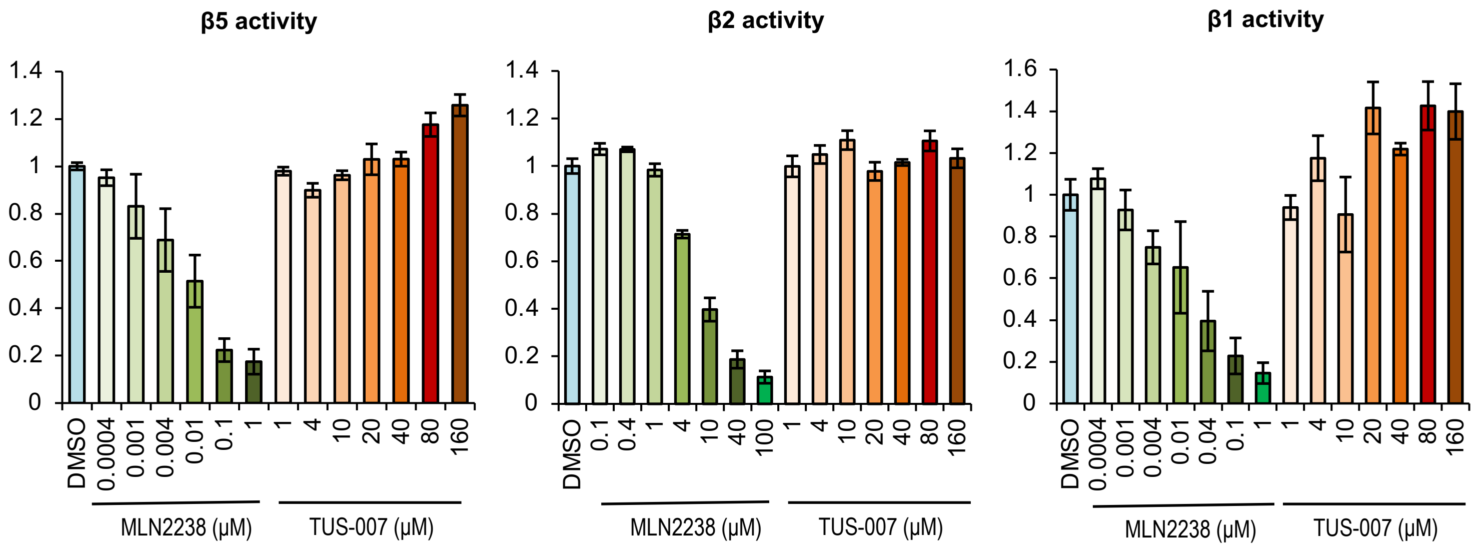


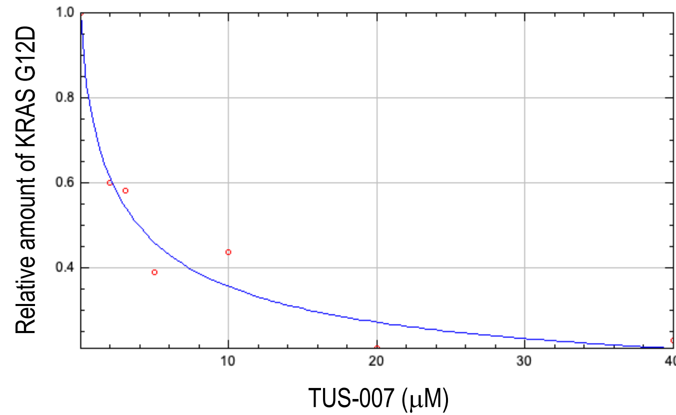
	T_m (°C)
DMSO	48.8 ± 0.05
RAS-SOS inhibitor	50.1 ± 0.15*
TUS-007	51.5 ± 0.2*,#

Supplementary Fig. 1. Estimation of T_m value of KRAS G12D incubated with TUS-007.
KRAS G12D was mixed with DMSO, RAS-SOS inhibitor (4 μM) or TUS-007 (4 μM) and incubated under heating from 25 °C to 99 °C. The denature of KRAS G12D was monitored by the fluorescence. The typical curve of each group was shown in upper panels. The means of T_m values were shown in lower table (mean ± SEM; n = 2). * $P < 0.05$ vs. DMSO, # $P < 0.05$ vs. RAS-SOS inhibitor.



Supplementary Fig. 2. Effects of TUS-007 on proteasome activity levels.
The levels of chymotrypsin-like (β5), trypsin-like (β2), and caspase-like (β1) proteasome activities was monitored by Suc-LLVY-AMC, Bz-VGR-AMC, and Z-LLE-AMC, respectively. AMC fluorescence was monitored by a plate reader with excitation and emission filters of 360 and 460 nm, respectively (DMSO, 30 min = 1) (mean ± SEM; n = 2-3).

a



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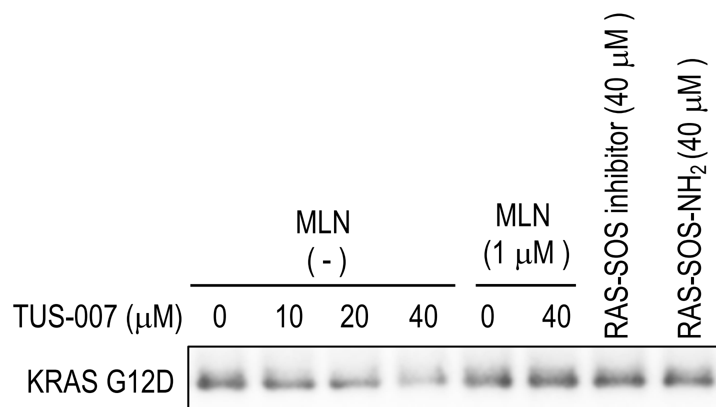

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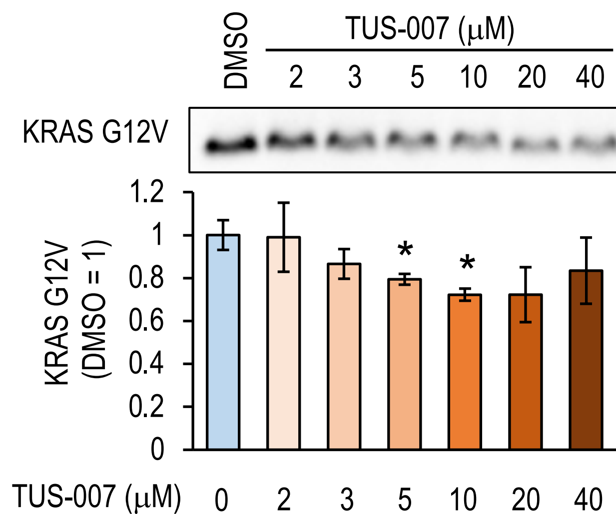
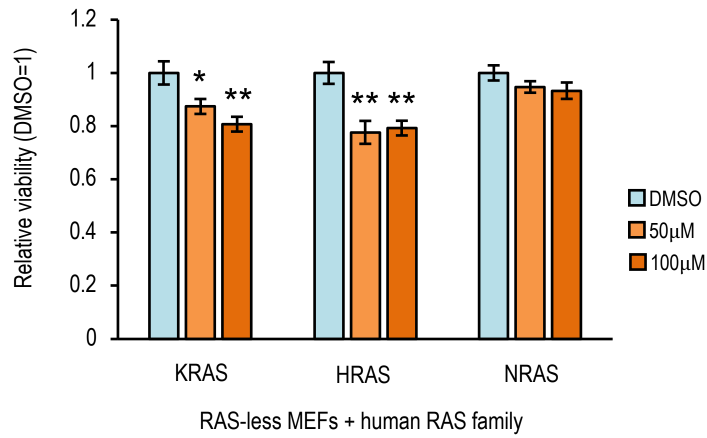


Supplementary Fig. 3. TUS-007 induced degradation of KRAS G12D/V in cell free assay.
a, Evaluation of the correlation between relative amount of KRAS G12D and concentration of TUS-007 were approximated with Rodbard. The DC50 was estimated about 4 μM. **b,** A proteasome inhibitor MLN2238 repressed KRAS G12D chemical knockdown by 26S proteasome. RAS-SOS inhibitor and RAS-SOS NH_2 did not induce KRAS G12D chemical knockdown. KRAS G12D incubated with 26S proteasome and agents as indicated for 3 h. **c,** KRAS G12V protein level was lower after incubation with TUS-007 at the indicated concentrations in the presence of 26S proteasome for 1 h, indicating successful KRAS G12V degradation by TUS-007 (mean ± SEM; n = 3). * $P < 0.05$ vs. DMSO.

a



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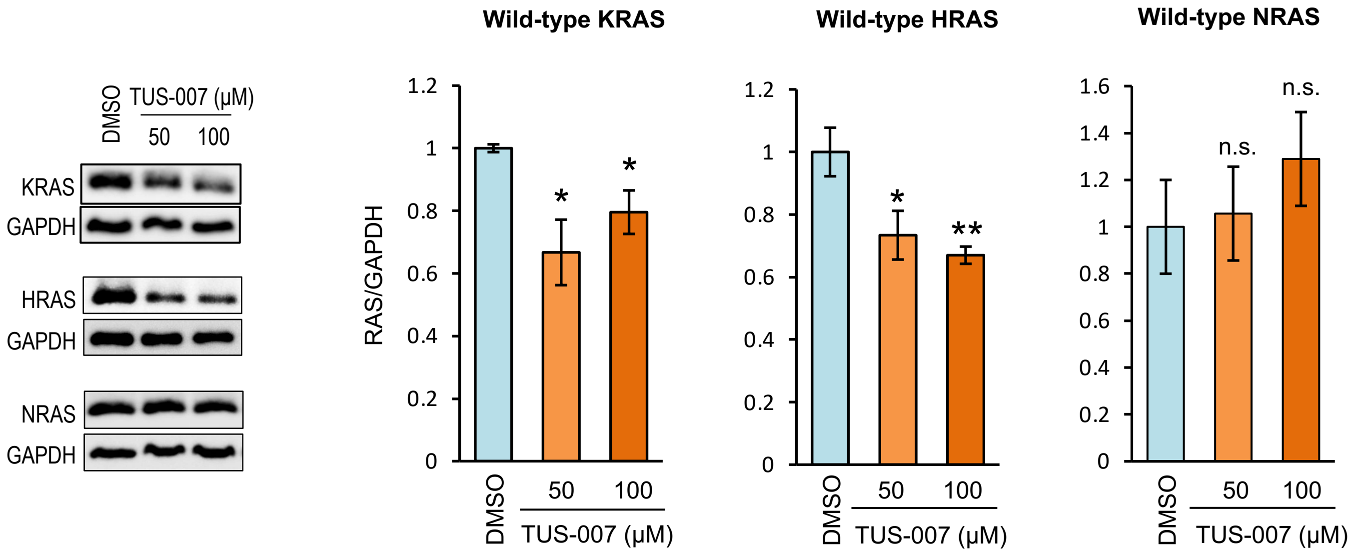
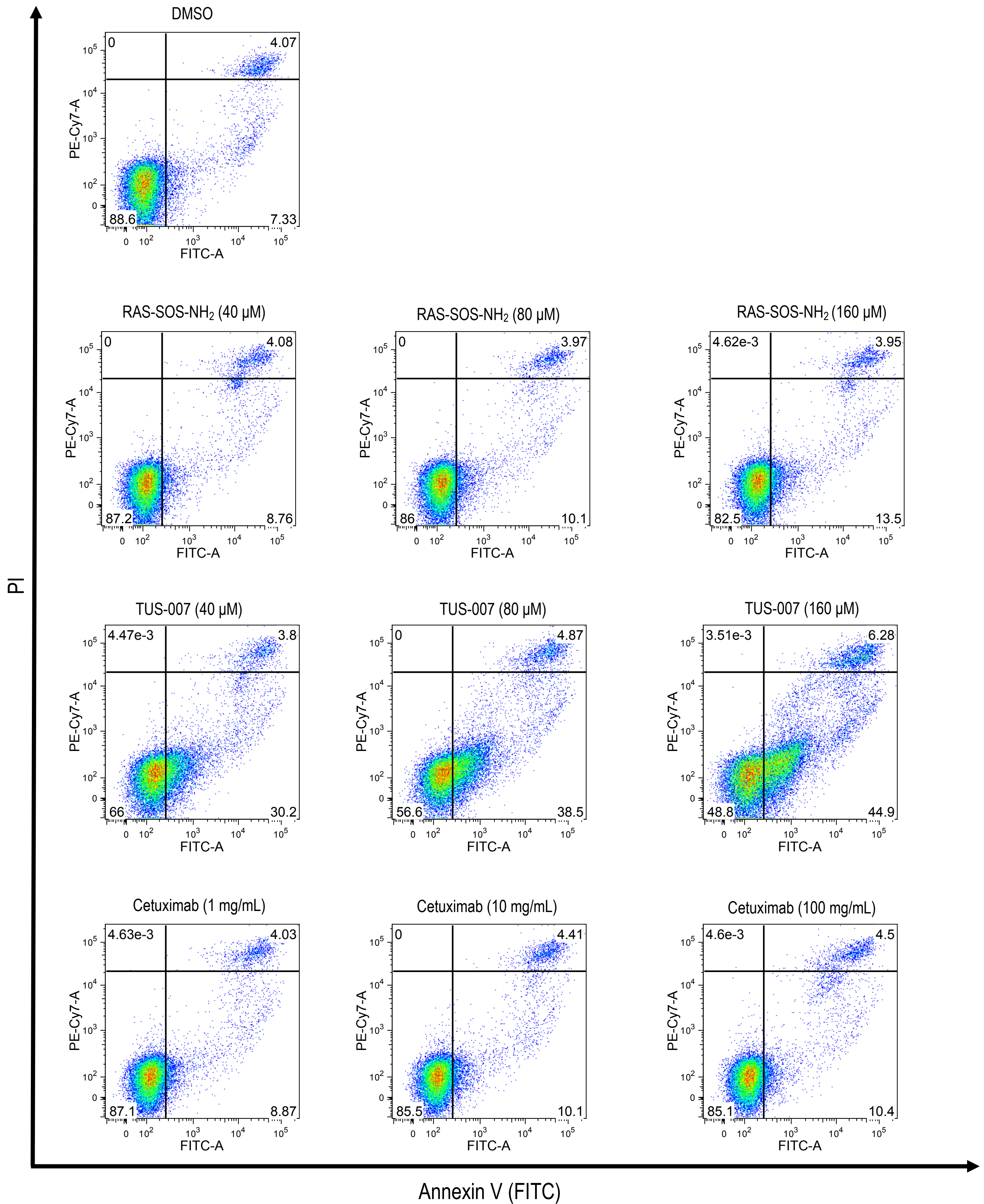
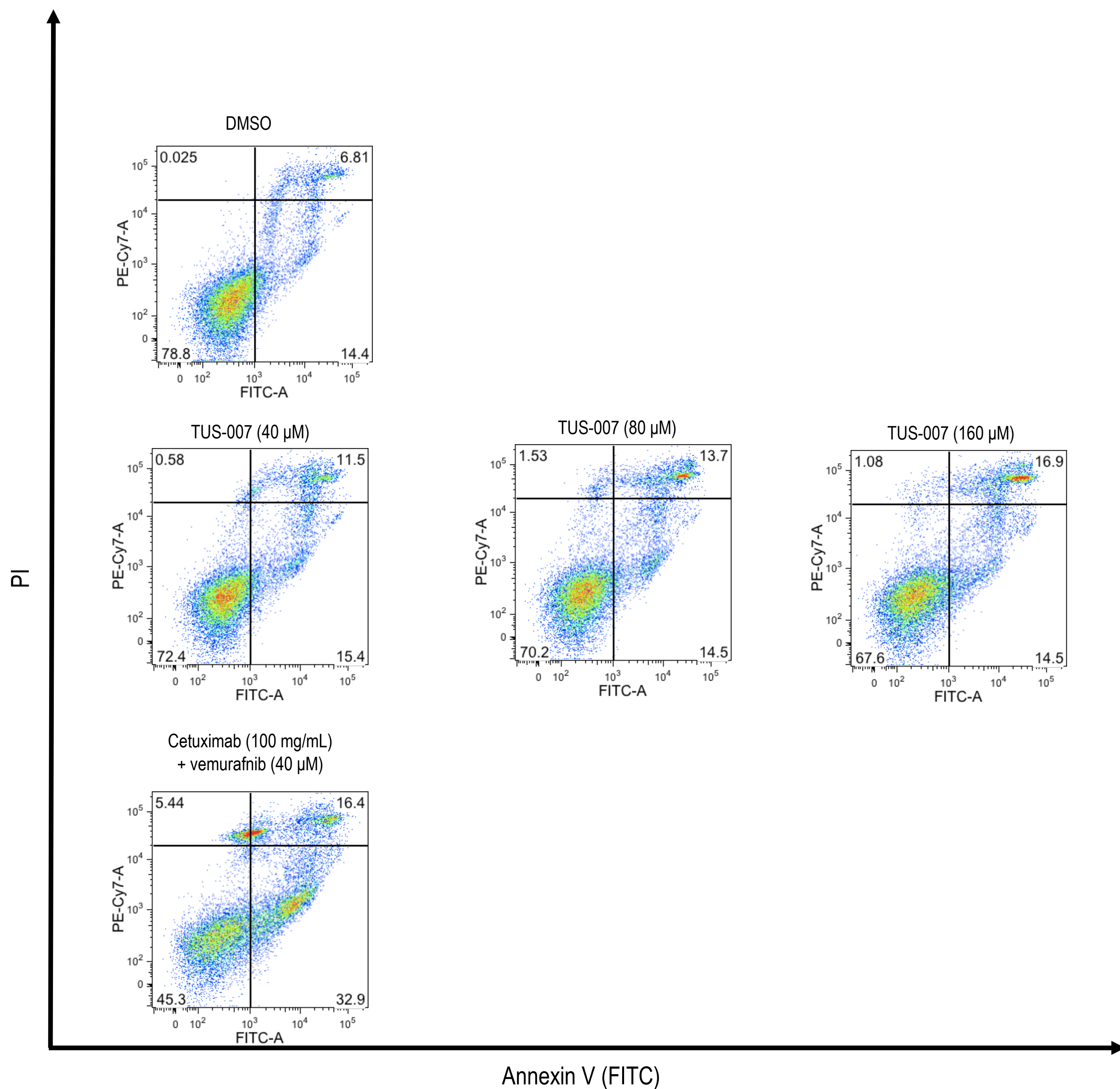


Supplementary Fig. 4. Selective chemical-knockdown of RAS variants in RAS-less MEFs expressing different types of human RAS.

a, Relative viability of RAS-less MEFs expressing WT human RAS family members after incubation with TUS-007 or DMSO. (mean ± SEM; n = 3-5). *$P < 0.05$ and **$P < 0.01$ vs. DMSO. **b,** Degradation of WT human RAS family members in RAS-less MEFs treated with TUS-007 or DMSO for 72 h (mean ± SEM; n = 4-5). *$P < 0.05$ and **$P < 0.01$ vs. DMSO.

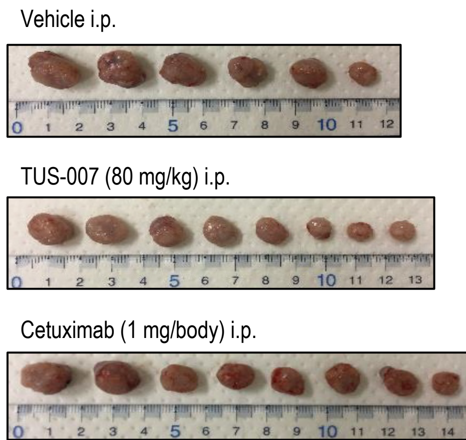


Supplementary Fig. 5. FACS plots for Annexin V- PI staining of SW620-Luc cells.
SW620-Luc cells were treated with the indicated agents for 48 h, followed by staining with Annexin V-FITC and PI. The typical plots are shown.



Supplementary Fig. 6. FACS plots for Annexin V- PI staining of HT-29-Luc cells.
HT-29-Luc cells were treated with the indicated agents for 48 h, followed by staining with Annexin V-FITC and PI. The typical plots are shown.

a



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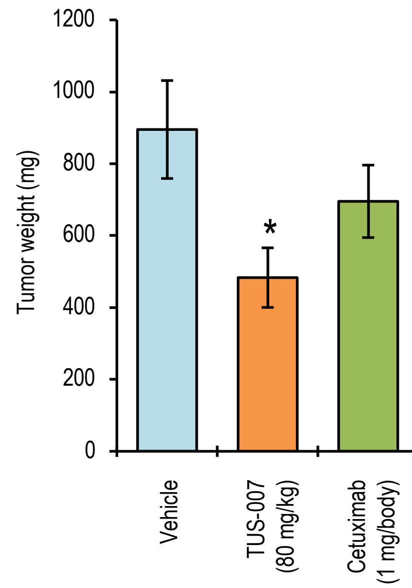

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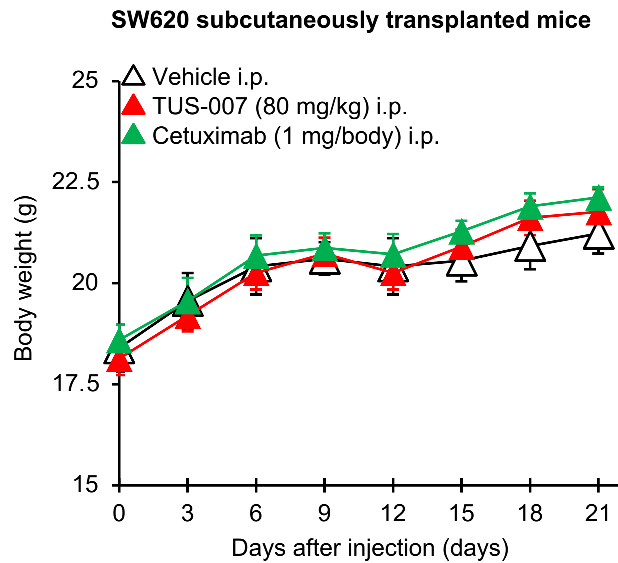
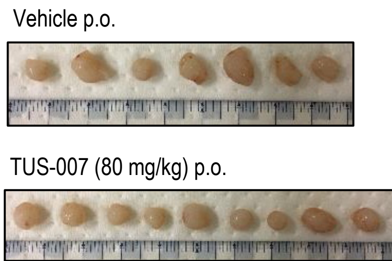


Supplementary Fig. 7. Effects of TUS-007 on the growth of colon cancer subcutaneous xenografts and toxicity.
a, Tumors of SW620-Luc cells at day 21 from the same mice shown in Fig. 2e (n = 6–8). **b,** Comparison of SW620-Luc tumor weight at 21 days after injection (mean ± SEM; n = 6–8). *P < 0.05 vs. vehicle alone. **c,** Body weight changes in mice with SW620-Luc cells transplanted subcutaneously (mean ± SEM; n = 6–8). Treatment with TUS-007 did not affect the body weight.

a



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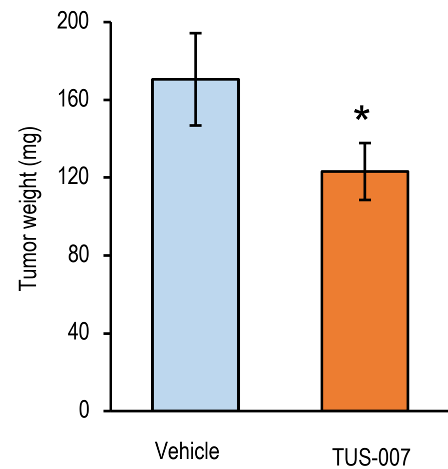

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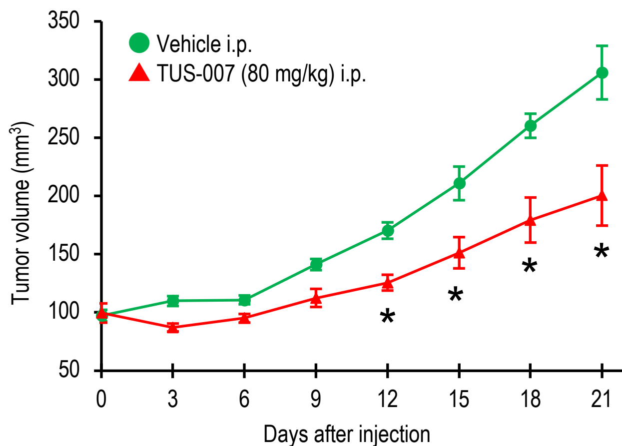

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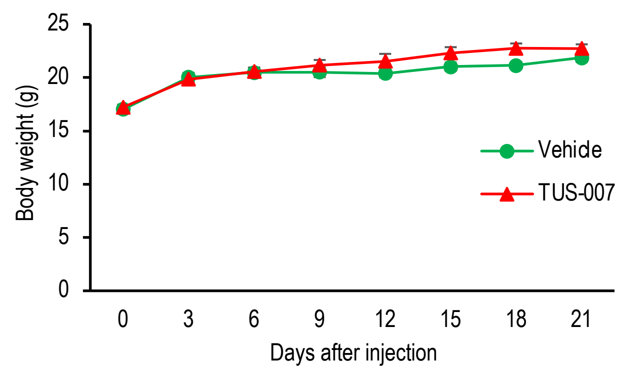
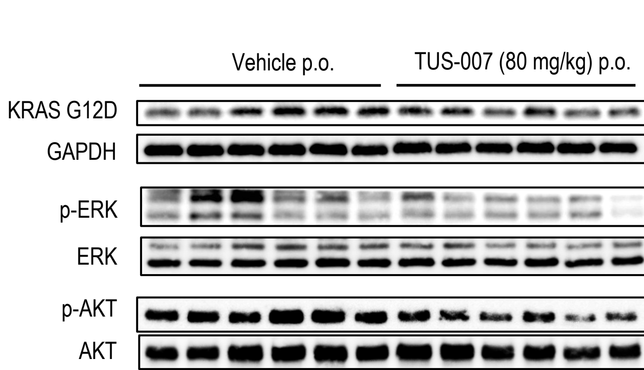
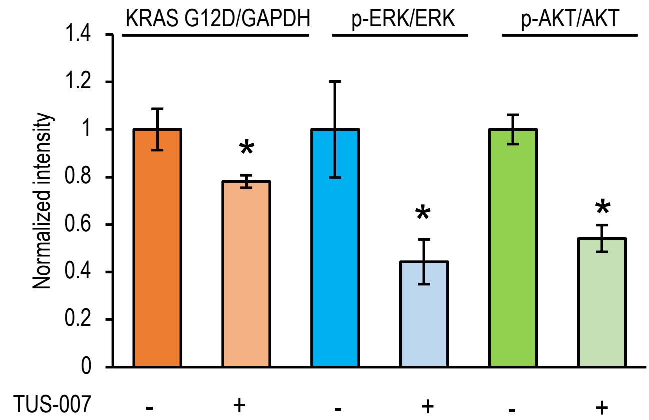


Supplementary Fig. 8. Effects of TUS-007 on the growth of pancreatic cancer subcutaneous xenografts and toxicity. **a,** Tumors of SW1990 cells from the same mice shown in Fig. 3e at 21 days after p.o. administration. **b,** Comparison of SW1990 tumor weight in **a** in this figure at 21 days after p.o. administration (mean ± SEM; n = 6-9). *P < 0.05 vs. vehicle alone. **c,** Tumor volume in mice with SW1990 cells transplanted subcutaneously and treated with i.p. administered TUS-007 or vehicle (mean ± SEM; n = 6-9). The agents were administered every three days. *P < 0.05 vs. vehicle. **d,** Body weight changes in mice with SW1990 cells transplanted subcutaneously (mean ± SEM; n = 6-9). i.p. treatment with TUS-007 did not affect the body weight.

a





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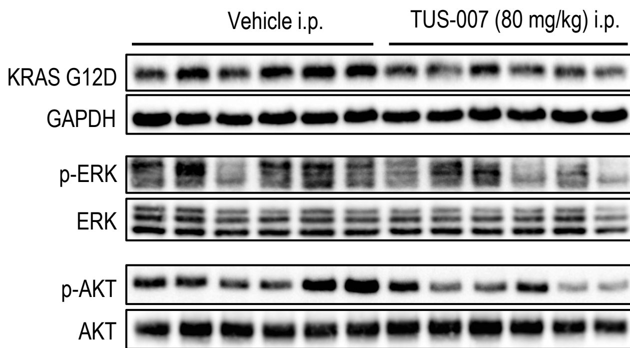
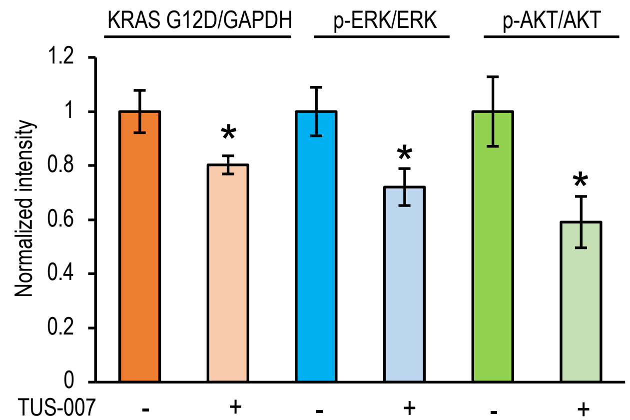
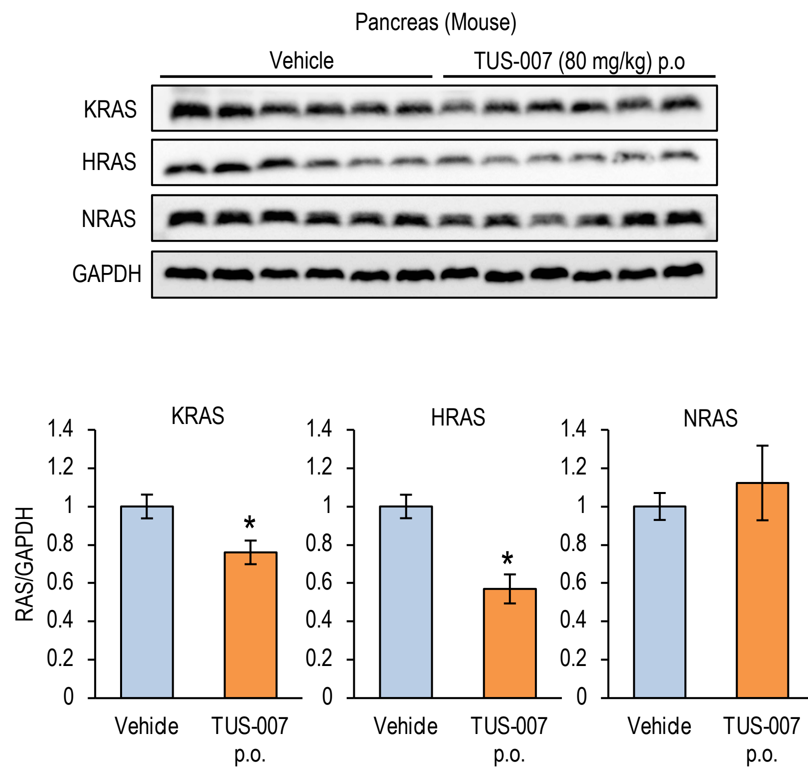
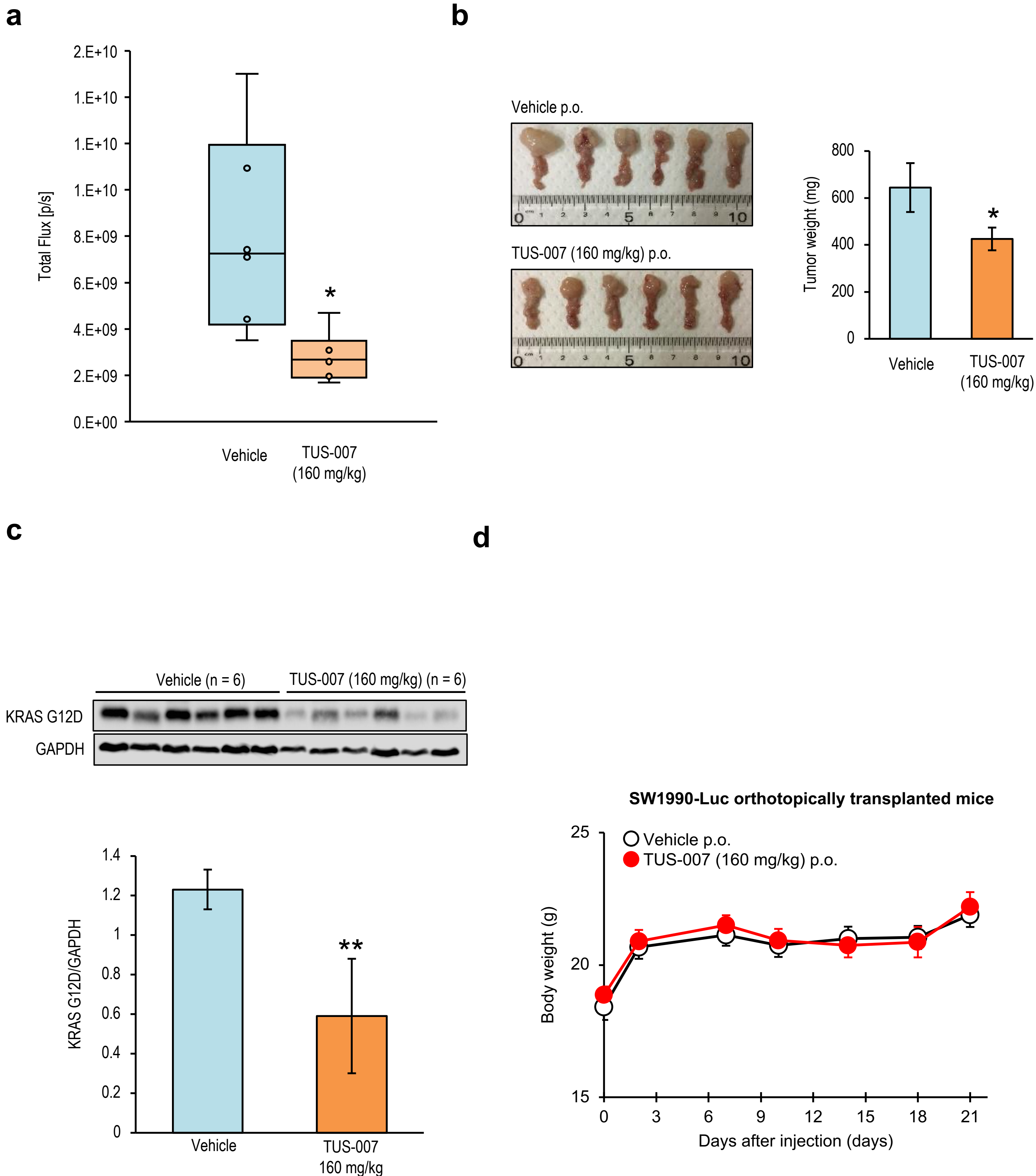




Supplementary Fig. 9. Effect of of TUS-007 on RAS signaling in of pancreatic cancer subcutaneous xenografts. . Immunoblotting of KRAS and downstream signaling molecules in tumors from the same mice used in Fig 3a (**a**: p.o treatment) and Extended Data Fig.8c (**b**: i.p. treatment). The quantitative analysis of the immunoblotting was shown as bar graph, where the KRAS values were normalized to GAPDH, the p-ERK values were normalized to the total ERK, and the p-AKT values were normalized to the total AKT (mean ± SEM; n = 6). *$P < 0.05$ vs. vehicle.



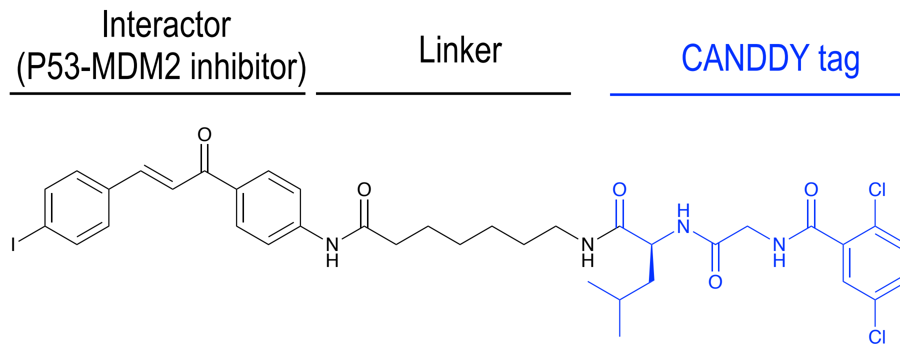
Supplementary Fig. 10. Effects of oral treatment with TUS-007 on wild type RAS in pancreas.
The immunoblotting analyses of wt RAS proteins in pancreas from the same mice shown in Fig. 3e. TUS-007 degraded KRAS and HRAS but not NRAS (mean ± SEM; n = 6-9). *P < 0.05 vs. vehicle alone.



Supplementary Fig. 11. Effect of TUS-007 on growth of orthotopic pancreatic cancer xenografts expressing mutant KRAS.

a, Tumor growth, as assessed by luciferase signal, in individual mice orthotopically transplanted SW1990-Luc cells treated with TUS-007 (red) or vehicle alone (blue) (mean ± SEM; n = 6). **b,** Pancreases from orthotopic xenograft model mice at day 21 after treatment with TUS-007 (lower-left panel) or the vehicle alone (upper-left panel) and comparison of their weights (right graph) (mean ± SEM; n = 6). *P < 0.05 vs. vehicle alone. **c,** Immunoblotting of KRAS G12D in tumor lysates from the same mice used in Fig. 4a. The bar graph shows quantification of KRAS G12D normalized to GAPDH (mean ± SEM; n = 6). **P < 0.01 vs. vehicle alone. **d**, Body weight changes in mice subjected to orthotopical transplantation of SW1990-Luc cells (mean ± SEM; n = 6). Treatment with TUS-007 did not affect the body weight.

a



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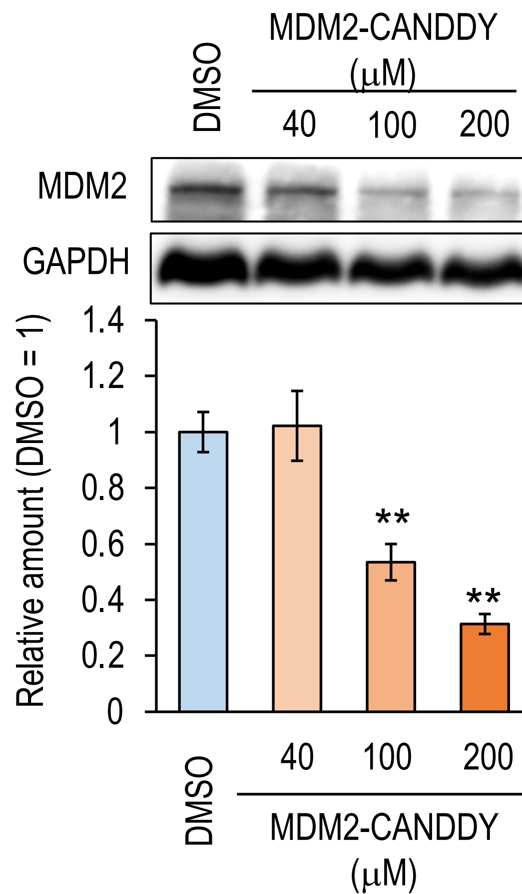

Supplementary Fig. 12. CANDDY induced degradation of MDM2, a common undruggable target.
a, The structure of MDM2-CANDDY using P53-MDM2 PPI inhibitor, with IC50 value between 6-25 μM. **b,** The human colon cancer cells HCT-116 were incubated for 48 h with MDM2-CANDDY. MDM2-CANDDY degraded MDM2 in the dose dependent manner (mean ± SEM. n = 3). ** P < 0.01 vs. DMSO.

Supplementary Table 1. **The concentration of TUS-007 in pancreas was maintained for 72h.**
The concentration of TUS-007 in pancreas from healthy wild-type mice i.p. treated with TUS-007 at 80 mg/kg (means ± SE, n = 5). The samples were obtained at indicated time points after i.p. injection. The concentration of TUS-007 was measured with HPLC.

Hours after i.p	1h	20h	72h
TUS-007 in pancreas (ng/mg)	45.7 ± 20.3	55.0 ± 28.6	61.8 ± 30.0