Supplementary Fig. 1. The TCR tetramer stains cells presenting 5-OP-RU but not 6-FP.
BEAS-2B.MR1-GFP cells were incubated with 10.5 uM 5-OP-RU, 100 uM 6-FP, or the equivalent volume of 0.01 M NaOH overnight in 6-well plates. Cells were then harvested and surface stained with APC-conjugated TCR tetramer or APC-conjugated anti-MR1 antibody. Data in Supplementary Fig. 1 and 2 were collected together and are representative of three independent experiments. The same isotype control samples are shown for TCR and antibody staining.
Supplementary Fig. 2. BEAS-2B MR1⁻/⁻ cells transfected with MR1 R9H are unable to present 5-OP-RU.

BEAS-2B MR1⁻/⁻ cells were transfected with the constructs encoding MR1 or MR1 R9H upstream of an IRES GFP sequence as in Fig. 3. 1e5 cells were incubated with 10.5 uM 5-OP-RU, 100 uM 6-FP, or the equivalent volume of 0.01 M NaOH overnight in 6-well plates. Cells were then harvested and surface stained with APC-conjugated TCR tetramer or APC-conjugated anti-MR1 antibody. Data in Supplementary Fig. 1 and 2 were collected together and are representative of three independent experiments. The same isotype control samples are shown for TCR and antibody staining.
Supplementary Fig. 3. Transfection efficiencies are not lower for MR1 R9H than MR1.
BEAS-2B MR1/- cells were transfected with the constructs encoding MR1 or MR1 R9H upstream of an IRES GFP sequence as in Fig. 3 and analyzed for GFP expression by flow cytometry. A representative dot plot with gating is shown on the left and percentages are pooled from eight different experiments including those shown in Fig. 3 and Supplementary Fig. 2. Bars denote median. Cell number and additional staining differ between experiments.
Supplementary Fig. 4. Specificity of Tetramer signal in Imaging.
BEAS-2B cells (1e5) were seeded on chamber slide and incubated for 18 h at 37C. Control wells received no tetramer. All cells then had CellMask Orange Plasma (1:5000 dilution, Invitrogen) added for 10 min before DAPI was added and cells were washed 2X in PBS. All images acquired were using the same microscope settings. Data are representative of three independent experiments.