# **SUPPLEMENTAL FIGURE LEGENDS**

# **Figure S1. Effects of multiple amino acid dropout on bovine blastocyst development.** Effect of BSA- versus amino acid-free culture media on development into blastocysts (B) of morphological grade 1-3 (B1-3) and B1-2 on Day (D) 8. Embryos were developing from D5 onwards under group (A) and single culture conditions without (B) or with (C) being washed before transfer into amino acid dropout medium. Asterisks indicate significant differences (\*=P<0.05, \*\*=P<0.001) from the complete protein control group. Error bars = sem; N = no. of embryos placed into IVC; n = no. of independent IVF experiments; Effect of dropping out (D) two to three (-IL, -IK, -KL, -IKL), (E) six (-HPRVWY, -CIKLMT) or (F) nine (+CMT, +IKL) candidate EAAs on blastocyst development into B1-3 and B1-2. ab= groups with different letters within each grading category differ P<0.005. Error bars = sem; n = no. of independent IVF experiments (N = no. of embryos placed into IVC); NEAAs = non-essential AAs. Dropped out amino acids are shown in 3 letter code.

# **Figure S2. TDH expression in mouse cells.** A) qRT-PCR for *TDH* gene expression in murine embryonic cells. Relative values were normalised on *GAPDH* expression. Error bars = sem. \*\* = Groups differ P<0.01 from the embryonic stem cell (ESC) control group; MEF = mouse embryonic fibroblasts; n = no. of independent biological replicates. B) Immunofluorescence staining of endogenous TDH in ESCs. Images were pseudo-colored using ImageJ. DNA was stained with Hoechst 33342 (blue), TDH was stained with anti-C-MYC primary and Alexa Fluor® 488 (green) secondary antibody. In the “2nd Ab” panel, primary antibody incubation was omitted. Scale bar = 100 μm. C) TDH immunoblot of murine induced pluripotent stem cells (miPSCs), MEFs and adult muscle fibroblasts (MFs). Histone 3 (H3) was used as a loading control.

# **Figure S3. *TDH*-overexpressing transgenic bovine cell line.** A) Alignment of mouse immunogenic peptide sequences that were used to raise anti-TDH polyclonal antisera against the bovine TDH sequence. Black boxes (white font) indicate identical amino acids whereas grey boxes (black font) indicate different ones between the mouse TDH-derived peptides and the bovine TDH sequence. B) Schematic of the *piggybac* plasmid used for Dox-inducible TDH overexpression under control of the modified tetracycline response element (TRE3G). TDH carries a C-terminal Myc-tag and is co-expressed with puromycin-resistance via an internal ribosome entry site (IRES). C) Immunostaining against TDH and C-MYC in BEF5 cells transiently transfected with Dox-inducible *TDH\_MYC* and analysed 48 h after transfection. I = induced with Dox for two days, NI = non-induced; anti-TDH polyclonal antisera (green) and anti-C-MYC monoclonal antisera (red). Images were pseudo-coloured using ImageJ software. D) Immunostaining against TDH in stable bovine BEF5-TDH\_MYC cells. BEF5-TDH = bovine embryonic fibroblast clonal line 5 overexpressing a Dox-inducible form of full-length *TDH\_MYC*; I = induced with Dox for two days, NI = non-induced; anti-TDH polyclonal antisera (green) and mitotracker (red). Images were pseudo-coloured using ImageJ software.

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# **Figure S4.** **TDH inhibition abrogates mouse blastocyst development.** A) Bright field images of mouse embryos treated with TDH inhibitor (Qc1) vs DMSO (vehicle control). B) Effect of Qc1 on mouse blastocyst development. N = no. of mouse embryos placed into treatment groups. N = no. of independent biological replicates. \*\* = Groups differ P<0.005, from the DMSO control group.

# **Figure S5. Morphological changes induced by TDH inhibition.** IVF embryos were cultured in medium containing DMSO (vehicle control) versus Qc1 from the morula-stage (D5) onwards. On D8, grade 1-2 expanded blastocysts were differentially stained. Blue (Hoechst 33342) and pink (propidium iodide) colours were designated as ICM and TE, respectively. Scale bar = 200 µm.