Supplementary Figures

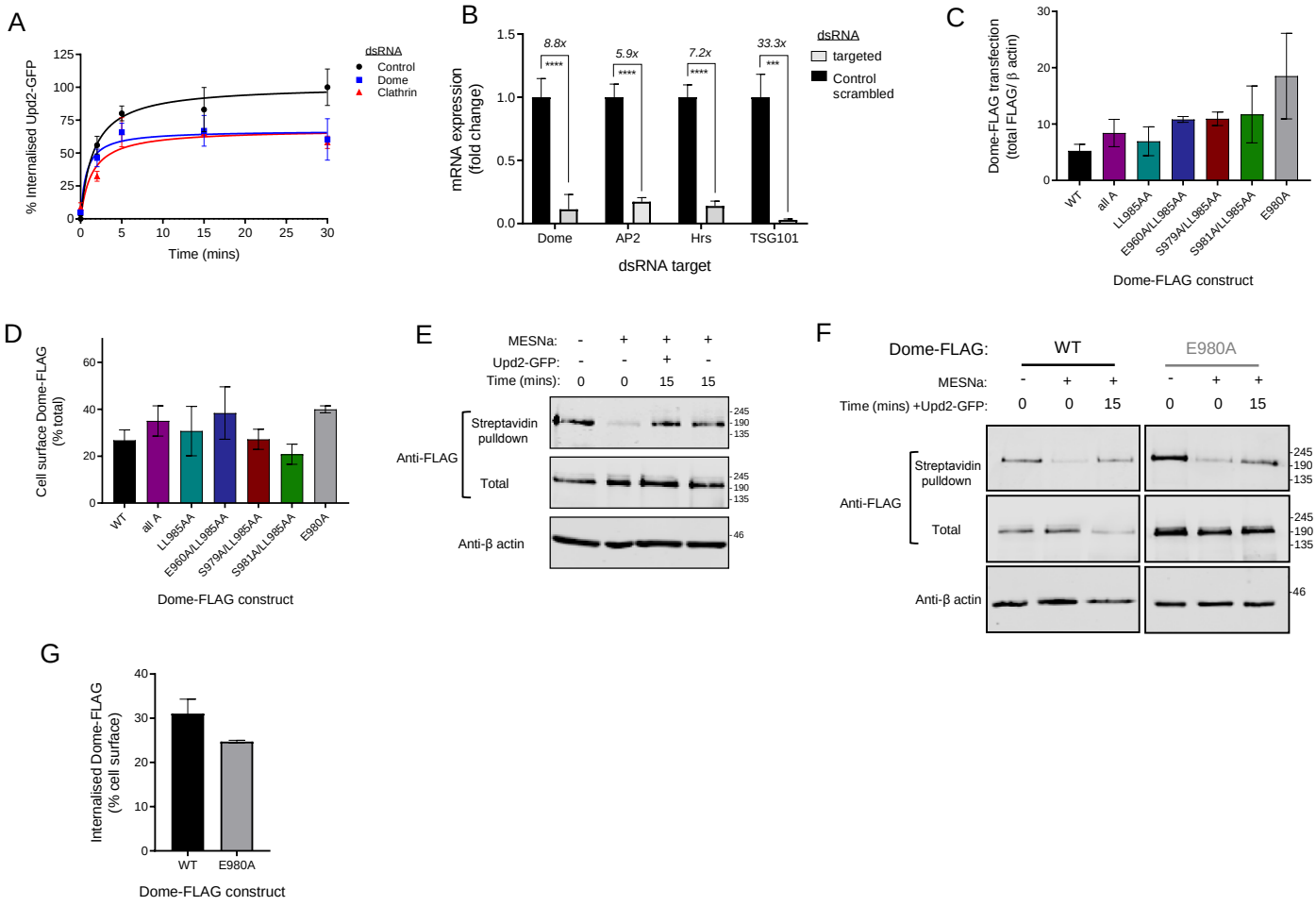

Figure S1

(A) CME is the route of GFP-Upd2 uptake at low ligand concentrations. S2R+ cells were treated for 5 days with control, clathrin (CHC) or Dome dsRNA. Cells were incubated with 3 nM Upd2-GFP for indicated time points at 25°C. Following acid washes, cell lysates were analysed with an anti-GFP ELISA. Internalised Upd2-GFP is expressed as percentage of the total amount internalised at 30 minutes. Graph is a representative experiment where each point is mean of triplicates +/- s.d. **(B)** mRNA levels of dsRNA targets following knockdown. S2R+ cells were treated with dsRNA 5 days prior to TRIzol RNA extraction. mRNA levels were analysed using qPCR, with levels of target mRNA normalised to rpl32 mRNA. Ratios are plotted as fold change compared to control dsRNA for each target mRNA. Graph represents the mean of triplicates +/- s.d. for at least 2 independent experiments (Dome = 2 repeats), or mean +/- s.e.m. for at least three independent experiments (AP2, Hrs and TSG101). Parametric, unpaired student's t-test was performed to compare control knockdown with targeted dsRNA knockdown, with ***p≤0.001, ****p≤0.0001. **(C)** Lysates from S2R+ cells transfected with FLAG-tagged Dome wild-type and mutants were prepared and subjected to SDS-PAGE and Western blotting with antibodies to FLAG and b-actin. The ratio of transfected Dome-FLAG construct is expressed as a function of the amount of b-actin. Graph is the mean ± s.d. of at least 2 independent experiments. Using student's t-test, there was no statistical difference between wild-type and mutant constructs. **(D)** Percentage of biotinylated Dome-FLAG at cell surface compared to total levels of transfected Dome-FLAG in cells expressing wild-type or mutant Dome-FLAG constructs. Using student's t-test, there was no statistical difference between wild-type and mutant constructs. **(E)** Dome is internalised efficiently in the absence of ligand. Sample immunoblot of cells transfected with Dome^{WT}-FLAG for 48hrs prior to cell surface biotinylation and endocytosis for 15 minutes +/- Upd2-GFP, followed by treatment +/- MESNa. Western blots were probed with antibodies as indicated. **(F)** Sample immunoblot of lysates from cells transfected with Dome^{WT}-FLAG or Dome^{E980A}-FLAG for 48 hrs prior to cell surface biotinylation and incubation at 25°C for times indicated +/- Upd2-GFP followed by treatment +/- MESNa. Western blots were probed with antibodies as indicated. **(G)** Quantitation of internalisation of Dome^{WT}-FLAG and Dome^{E980A}-FLAG. Percentage of cell-surface receptor that is internalised after 15 mins at 25°C. Background of biotinylated cell surface Dome-FLAG after 0 mins endocytosis and MESNa treatment was subtracted and internalised Dome-FLAG was then calculated as a percentage of total cell surface Dome-FLAG prior to MESNa treatment. Graphs represent mean +/- s.d. for 2 independent experiments and no significant differences were observed.

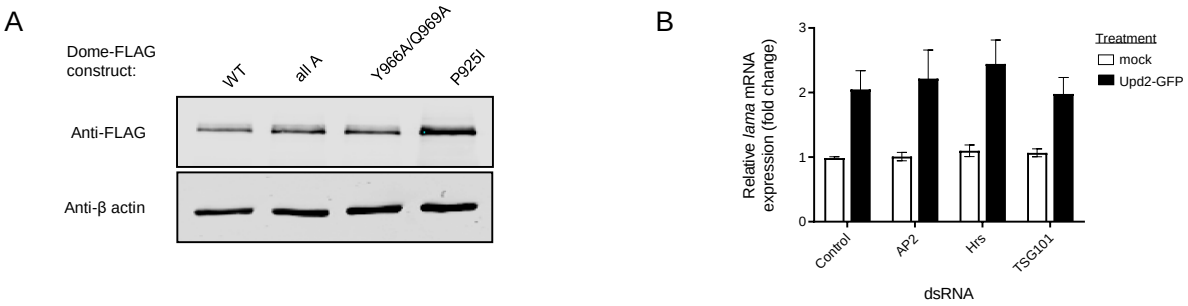

Figure S2:
(A) Sample immunoblot of relative transfection efficiencies of Dome^{WT}-FLAG, Dome^{allA}-FLAG, Dome$^{Y966A/Q969A}$-FLAG and Dome ^{P925I}-FLAG. Blots were probed with antibodies as indicated.
(B) *lama* expression is independent of endocytosis. S2R+ cells were treated with dsRNA against AP2, Hrs and TSG101 as well as non-targeting (control) dsRNA for 5 days. Cells were incubated with 3 nM Upd2-GFP for 2.5 hrs prior to RNA extraction. *lama* mRNA levels were normalised to that of reference gene Rpl32, and presented as fold change compared to mock-treated control samples. Results are expressed as means of triplicates +/- s.e.m. for 3 independent experiments.

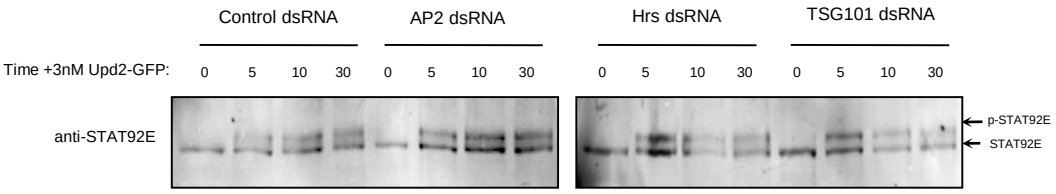

Figure S3: STAT92E phosphorylation is not regulated by endocytosis.
Representative immunoblot of control vs AP2, Hrs and TSG101 knockdown S2R+ cells treated with 3 nM Upd2-GFP at 25° C for the indicated times. Cells were treated with targeting dsRNA and incubated for 5 days at 25° C. Total protein extract was analysed by SDS-PAGE and immunoblotted with anti-STAT92E antibodies.

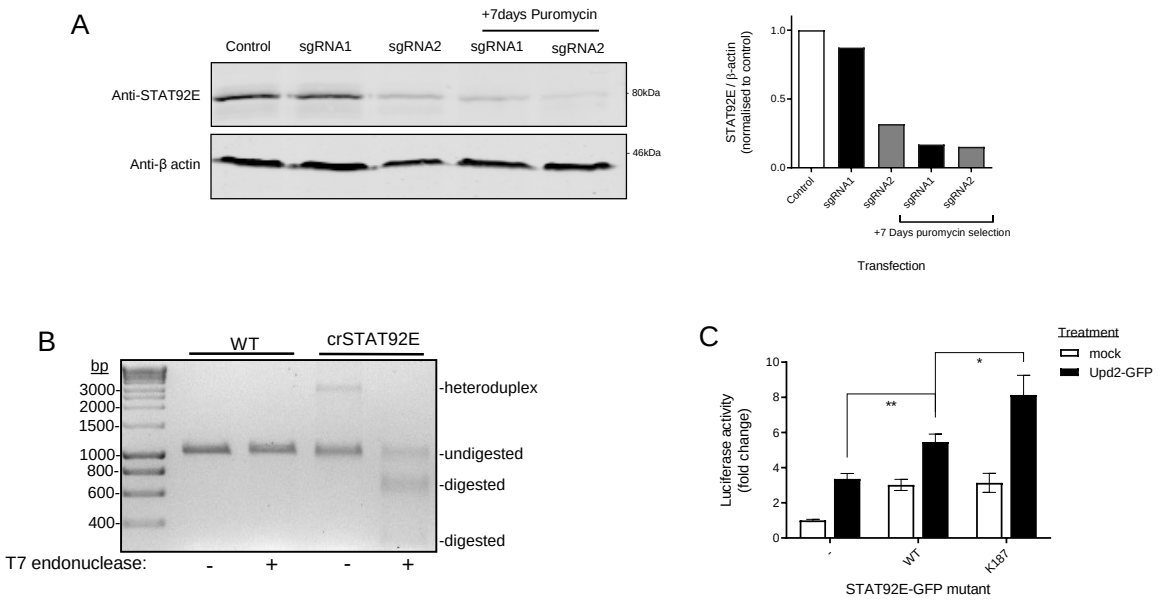

Figure S4: Generation and characterization of STAT92E negative S2R+ cells.
(A) Immunoblot and quantification demonstrating levels of STAT92E protein in cells transfected with pAc-sgRNA-Cas9 targeting STAT92E for 3 days, and then either with or without puromycin selection as indicated. Blots were probed with antibodies as indicated.
(B) T7-endonuclease assay demonstrates Cas9 induced mutation in the STAT92E gene. Genomic DNA was extracted from WT and crSTAT2 cell lines, and a 989bp region around the sgRNA target site was amplified by PCR. Addition of T7 endonuclease to the PCR product causes multiple bands for crSTAT2 cell line but not WT cells.
(C) Mutation of Lys187 increases STAT92E signalling. crSTAT cells were transfected with pAc-Ren, *10xSTAT-Luciferase* and pAc5.1 (-), $STAT92E^{WT}$-GFP or $STAT92E^{K187R}$-GFP. Cells were stimulated with 0.75 nM Upd2-GFP for 30 mins, then incubated in fresh media for 18 hrs followed by measurement of bioluminescence. Data is mean +/- s.e.m. from 3 independent experiments and normalised to cells transfected with pAc5.1 (-) and treated with 0 nM Upd2-GFP. *: $p<0.05$; **: $p<0.01$.

Table S1: SgRNA oligos

Oligo	Sequence
sgRNA1.1	TTCGACAACACGCCCATGGTTACC
sgRNA1.2	AACGGTAACCATGGGCGTGTTGTC
sgRNA2.1	TTCGACCATGTACCCGGTAACCAT
sgRNA2.2	AACATGGTTACCGGGTACATGGTC

Table S2: Primers for qPCR

Gene	CG number	Forward primer	Reverse primer
Rpl32	CG7939	GACGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG
domeless	CG14226	ACTTTCGGTACTCCATCAGC	TGGACTCCACCTTGATGAG
tsg101	CG9712	GAGGAGACACAAATAACAAAGTACC	TGAGTGTCCATCAACCAAATAC
clathrin heavy chain(CHC)	CG9012	GTAGTAAAGATGACGCAACCAC	GTTCATGTCAATGATGACCACT
α-adaptin	CG4260	ACCAGCGAAAATTAACAAGC	GAGACGACTTCACACCCTTC
socs36A	CG15154	AGTGCTTTACTGCTGCGACT	TCGTCGAGTATTGCGAAGT
lama	CG10645	TGATATTGCTGCTTTCCTGGAC	TGGTTTGGCGATGGTTTTAT