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nature cell biology

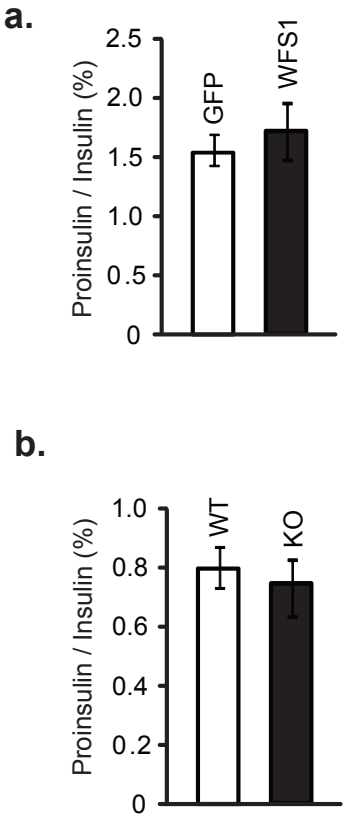

Figure S1 WFS1 does not modulate insulin processing. **a,** The ratio of proinsulin to insulin release was measured in rat islets transduced with GFP or WFS1 lentivirus and treated for 2 hr with 16.7 mM glucose (n=3). **b,** The ratio of proinsulin to insulin release was measured in islets from $Wfs1^{+/+}$ (WT) and $Wfs1^{-/-}$ (KO) mice and treated for 2 hr with 16.7 mM glucose (n=3). Data are means ± SD.

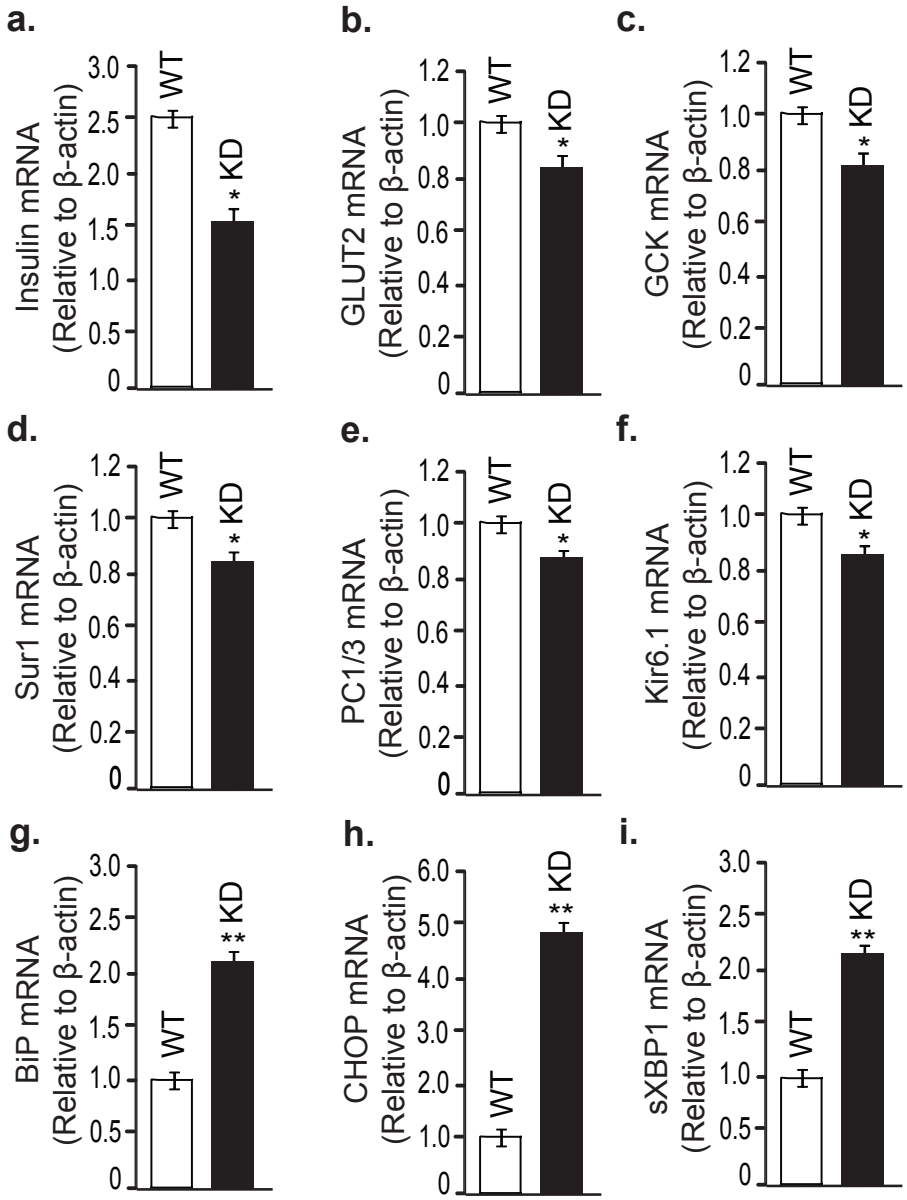

Figure S2 Glucose sensing genes are suppressed in WFS1 knockdown cells. **a-i,** Total mRNA was prepared from INS-1 832/13 pTetR with endogenous WFS1 (WT) or suppression of endogenous WFS1 with shRNA (KD) (n = 3). Relative expression levels of insulin **(a)**, glucose transporter 2 (GLUT2) **(b)**, glucokinase (GCK) **(c)**, sulfonylurea receptor 1 (Sur1) **(d)**, prohormone convertase (PC1/3) **(e)**, potassium inwardly rectifying K channel subunit (Kir6.1) **(f),** immunoglobulin heavy chain-binding protein (BiP) **(g)**, C/EBP homologous protein (CHOP) **(h),** and spliced X box-binding protein 1 (sXBP1) **(i)** were measured by qPCR (n = 3). Data are means ± SD. *p<0.05, **p<0.01.

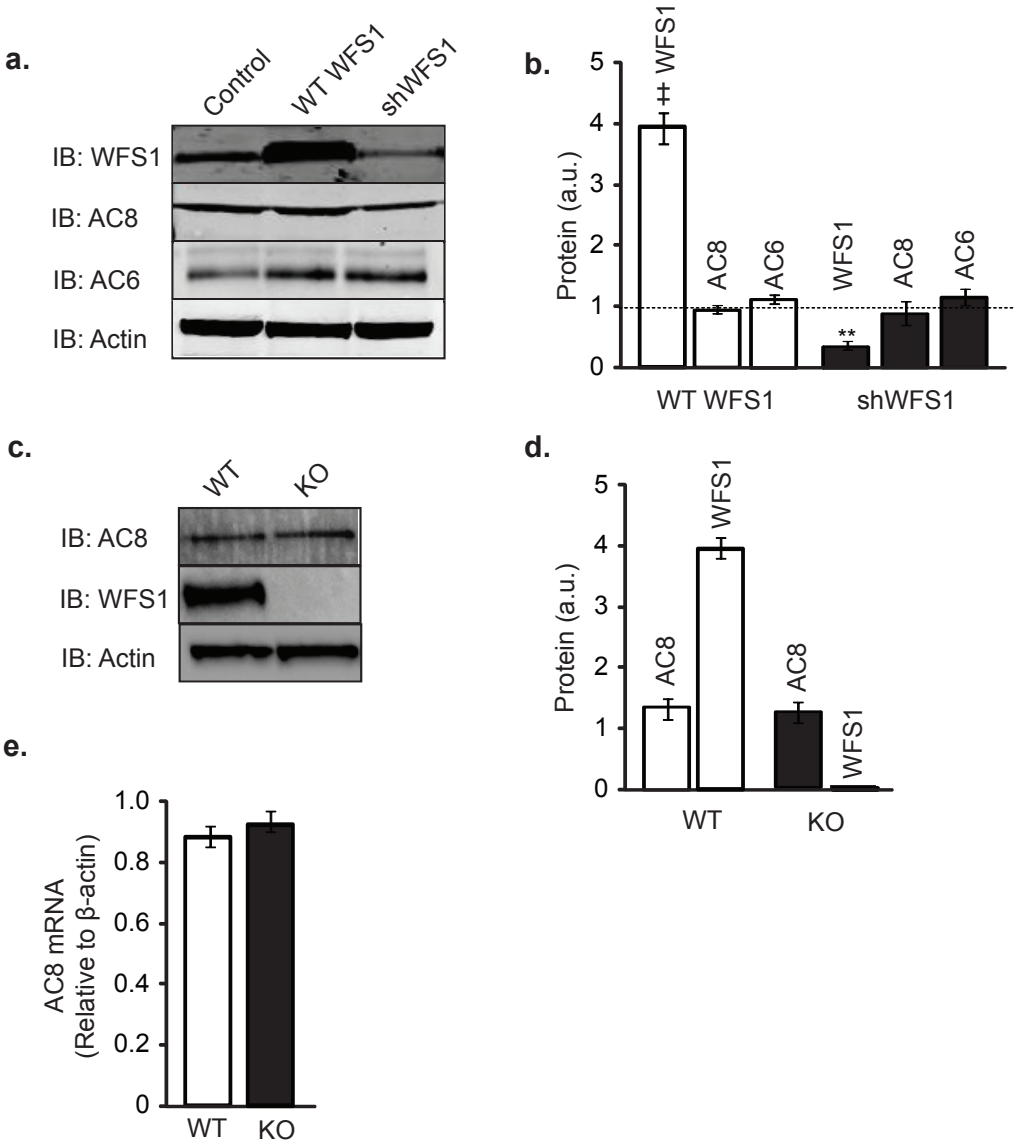

Figure S3 WFS1 does not regulate adenylyl cyclase 6 (AC6) or 8 (AC8) expresssion. **a,** Lysates from INS-1 832/13 pTetR cells with endogenous WFS1 expression (Control), overexpression of WFS1 (WT WFS1), and suppression of endogenous WFS1 (shWFS1) were immunoblotted (IB) with the following antibodies: anti-WFS1, anti-AC8, anti-AC6, and anti-actin. **b,** Protein levels of WT WFS1 and shWFS1 samples were quantified using ImageJ and normalized to control (dotted line) (n = 3) **c,** Lysates from Wfs1$^{+/+}$ (WT) and Wfs $^{-/-}$ (KO) islets were IB with the following antibodies: anti-WFS1, anti-AC8, and anti-actin. **d,** Protein levels of WT and KO samples were quantified using ImageJ (n = 3). **e,** Relative mRNA expression of AC8 was measured by qPCR in WT and KO islets (n = 3). Data are means ± SD. **p<0.01, ‡ p<0.001.

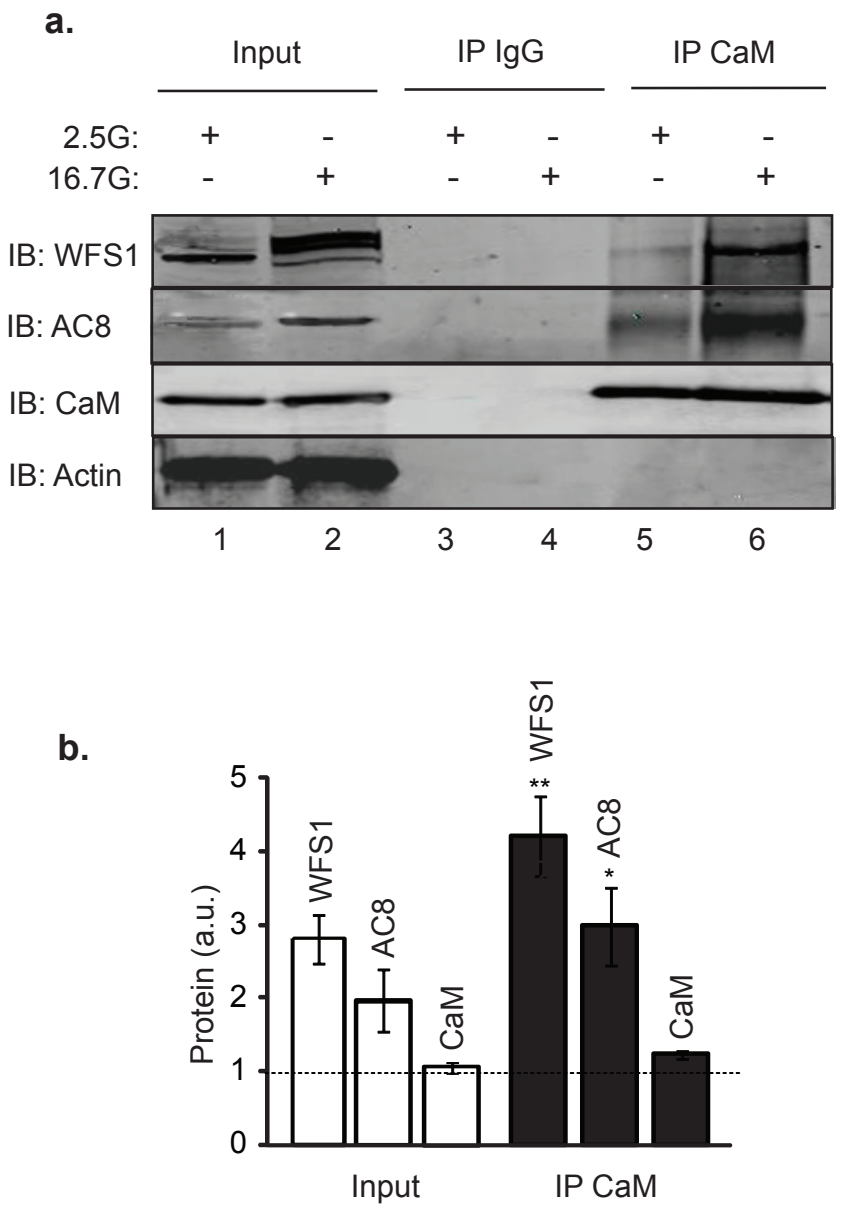

Figure S4 WFS1 forms a glucose-dependent complex with adenylyl cyclase 8 (AC8) and calmodulin (CaM). **a,** CaM was immunoprecipitated (IP) from cell lysates prepared from INS-1 832/13 cells treated with 2.5mM (2.5G) or 16.7 mM glucose (16.7G) for 2 hr. Immunoprecipitates were immunoblotted (IB) with anti-WFS1, anti-AC8, anti-CaM, and anti-actin antibodies. **b,** Protein levels of samples treated with 16.7G in (a) were quantified using ImageJ and normalized to 2.5G samples (dotted line) (n = 3). Data are means ± SD. *p<0.05, **p<0.01.

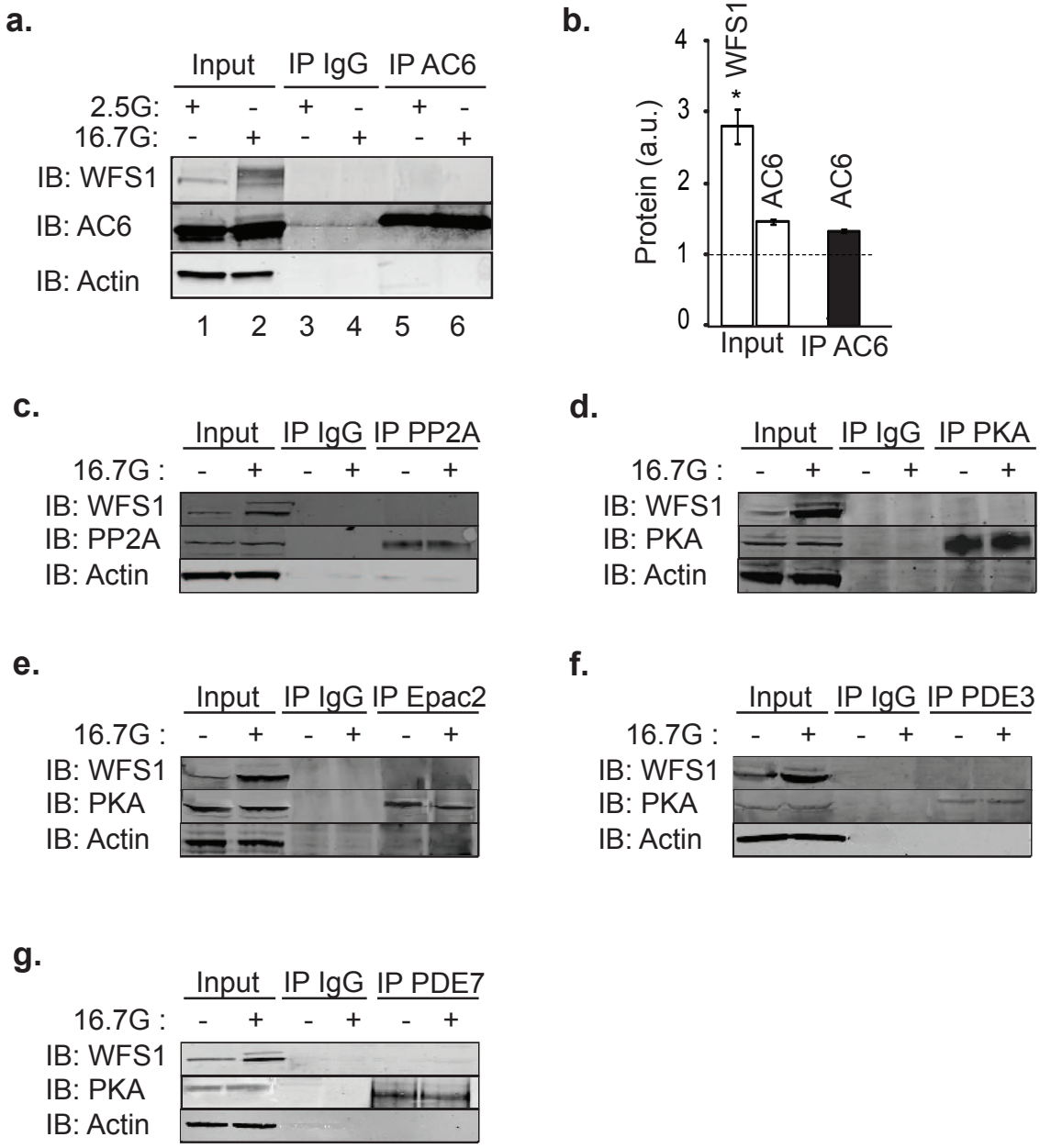

Figure S5 WFS1 does not form a complex with adenylyl cyclase 6 (AC6) or other cAMP pathway proteins **a,** AC6 was immunoprecipitated (IP) from cell lysates prepared from INS1 832/13 treated with 2.5 mM (2.5G) or 16.7 mM gluocse (16.7G) for 1 hr. Immunoprecipitates were immunoblotted with anti-WFS1, anti-AC6, and anti-actin antibodies. **b,** Quantification of input and IP proteins (bars) from (a) as a fold increase from 2.5G (dotted line) **c,** Protein phosphatase 2A (PP2A) was immunoprecipitated (IP) from cell lysates prepared from INS-1 832/13 cells, treated with 2.5 mM or 16.7 mM glucose (16.7G) for 2 hr. Immunoprecipitates were immunoblotted (IB) with anti-WFS1, anti-PP2A, and anti-actin antibodies. **d,** Protein kinase A (PKA) was IP from INS-1 832/13 cell lysates prepared as in (a). Immunoprecipitates were IB with anti-WFS1, anti-PKA, and anti-actin antibodies. **e,** Exchange protein directly activated by cAMP 2 (Epac2) was IP from INS-1 832/13 cell lysates prepared as in (a). Immunoprecipitates were IB with anti- WFS1, anti-Epac2, and anti-actin antibodies. **f,** Phoshodiesterase 3B (PDE3) was IP from INS-1 832/13 cell lysates prepared as in (a). Immunoprecipitates were IB with anti-WFS1, anti-PDE3, and anti-actin antibodies. **g,** Phoshodiesterase 7 (PDE7) was IP from INS-1 832/13 cell lysates prepared as in (a). Immunoprecipitates were IB with anti-WFS1, anti-PDE7, and anti-actin antibodies (n=3). Data are means ± SD. *p<0.05.

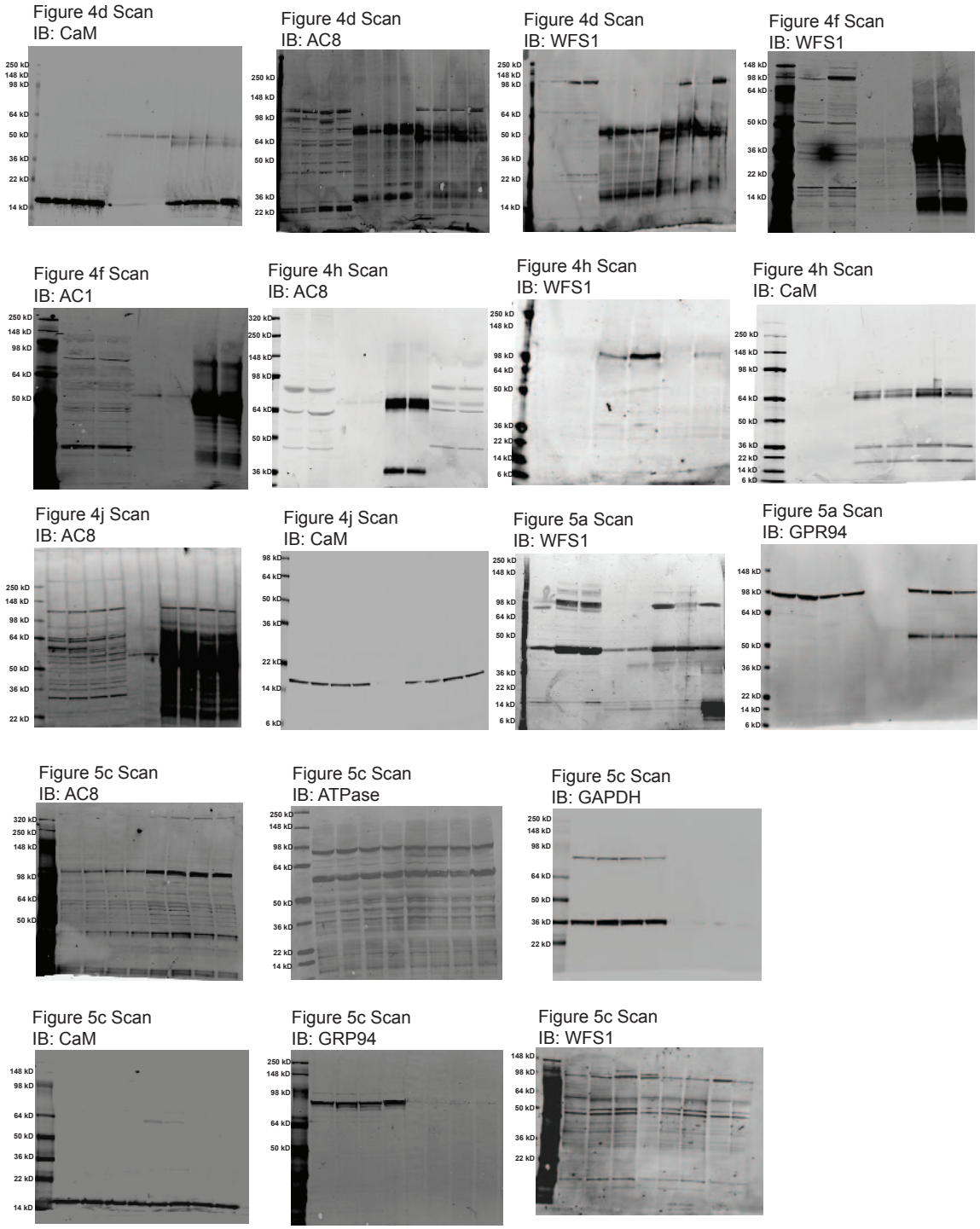

Figure S6. Full blot scans. Full immunoblot scans for Fig. 4d, 4f, 4h, 4j, 5a, and 5c.