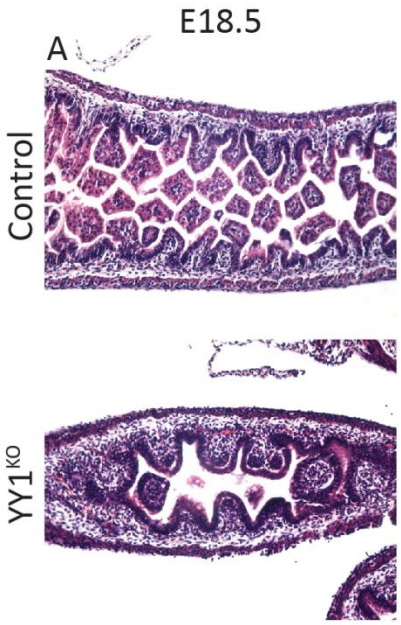


Fig. S1 **YY1 mutants have a villus development defect in the developing ileum**

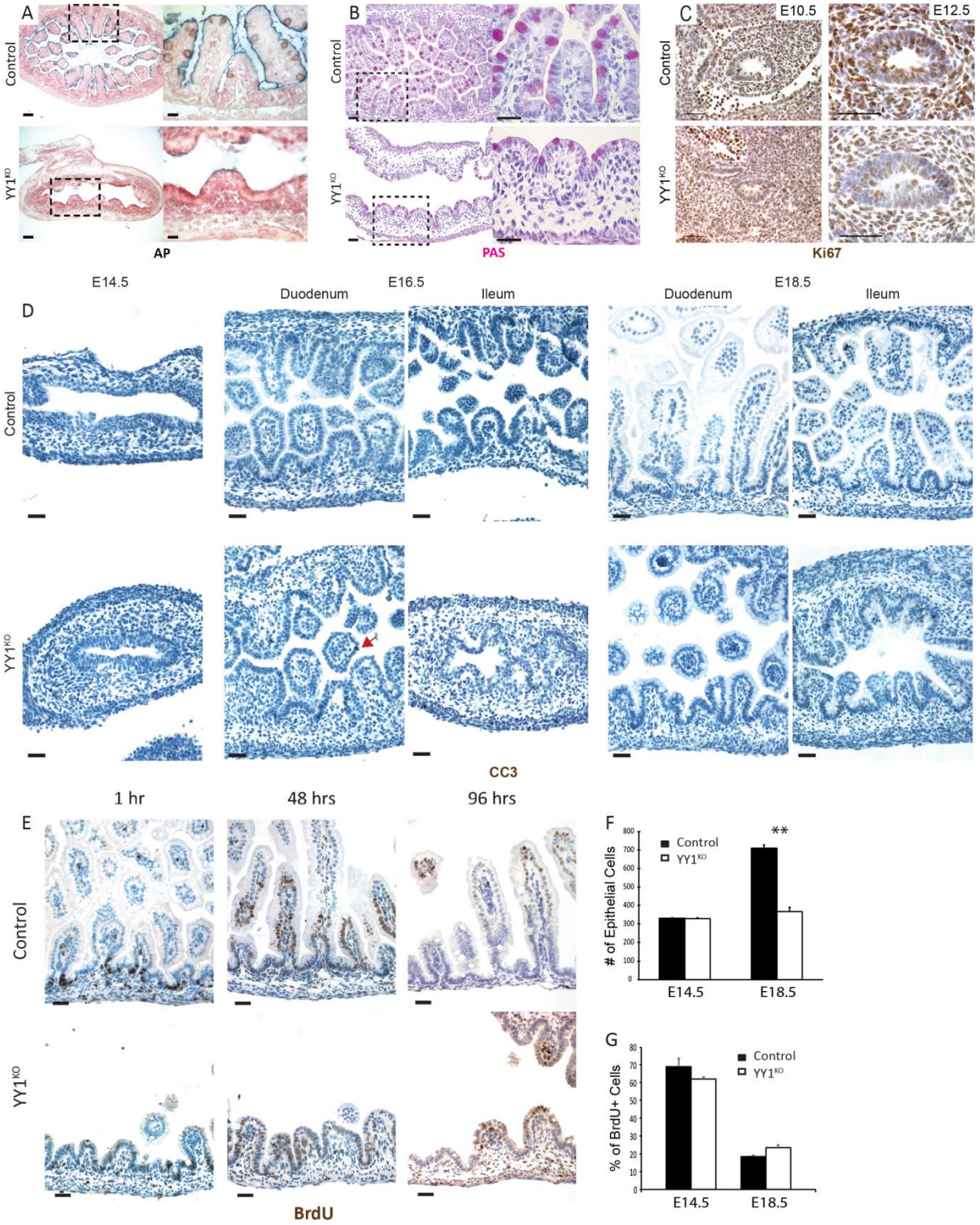


Fig. S2 **YY1 mutants do not have significant cell death, but have compromised enterocyte differentiation in ileum.**
(A) Yy1 mutant enterocytes exhibit diminished alkaline phosphatase staining in developing ileum. (B) YY1 mutants have similar goblet cell differentiation in ileum compared to littermate controls, as seen by PAS staining. (C) Ki67 immunohistochemistry shows no significant difference in proliferation at E10.5 and E12.5 (D) YY1 mutants do not have drastically higher cell death compared to littermate controls, as seen by CC3 staining. (E) Representative BrdU pulse chase immunostaining as documented in figure 2I. (F) Epithelial cell number is substantially reduced in the YY1 mutants at E18.5 (***P* value=2.78E^{-4},n=4) but no difference is seen at E14.5 (*P* value=0.95, n=4). Counts reflect number of epithelial cells per mm of tissue. (G) Similar numbers of BrdU positive cells were observed between controls and mutants in duodenum at E14.5 and E18.5. BrdU counts represent the percent of positive cells versus total epithelial cells counted. All analysis was done on duodenal tissue unless otherwise noted. Error bars show standard error ** indicates *P*value < 0.0

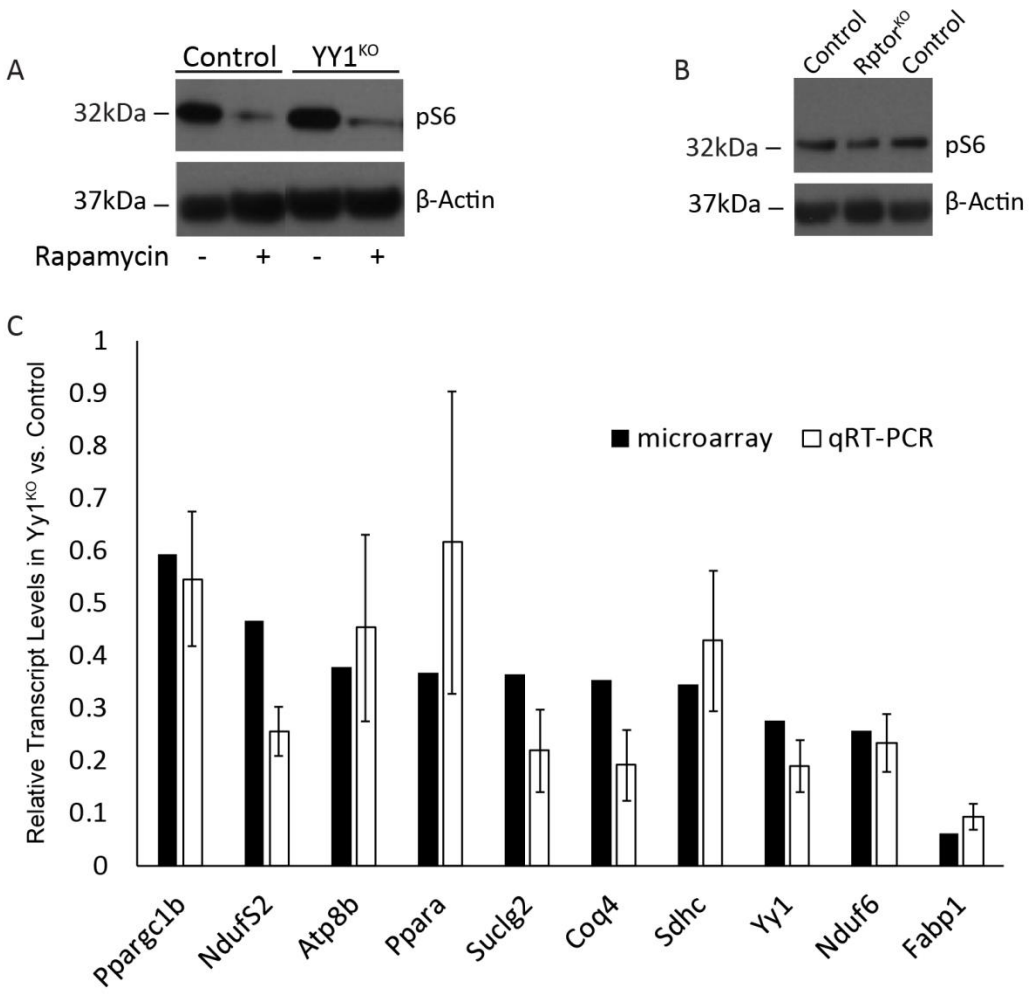


Fig. S3 **YY1 acts independently of mTOR signaling in villogenesis**

(A) Immunoblot showing diminished pS6 levels in isolated epithelial cells from mice treated with rapamycin. (B) pS6 levels were also diminished in whole tissue extracts from epithelial cell specific *Rptor* knockout. Levels of pS6 decrease do not appear as robust due to inclusion of non-epithelial cells in the protein lysate. (C) A set of genes was selected to validate the microarray findings by qRT-PCR. Shown is the relative transcript level compared to the matched control. For qRT-PCR, data were normalized to actin transcript levels, n=3 control and 5 knockout isolated epithelia from E18.5 intestine; Error bars indicate standard error.

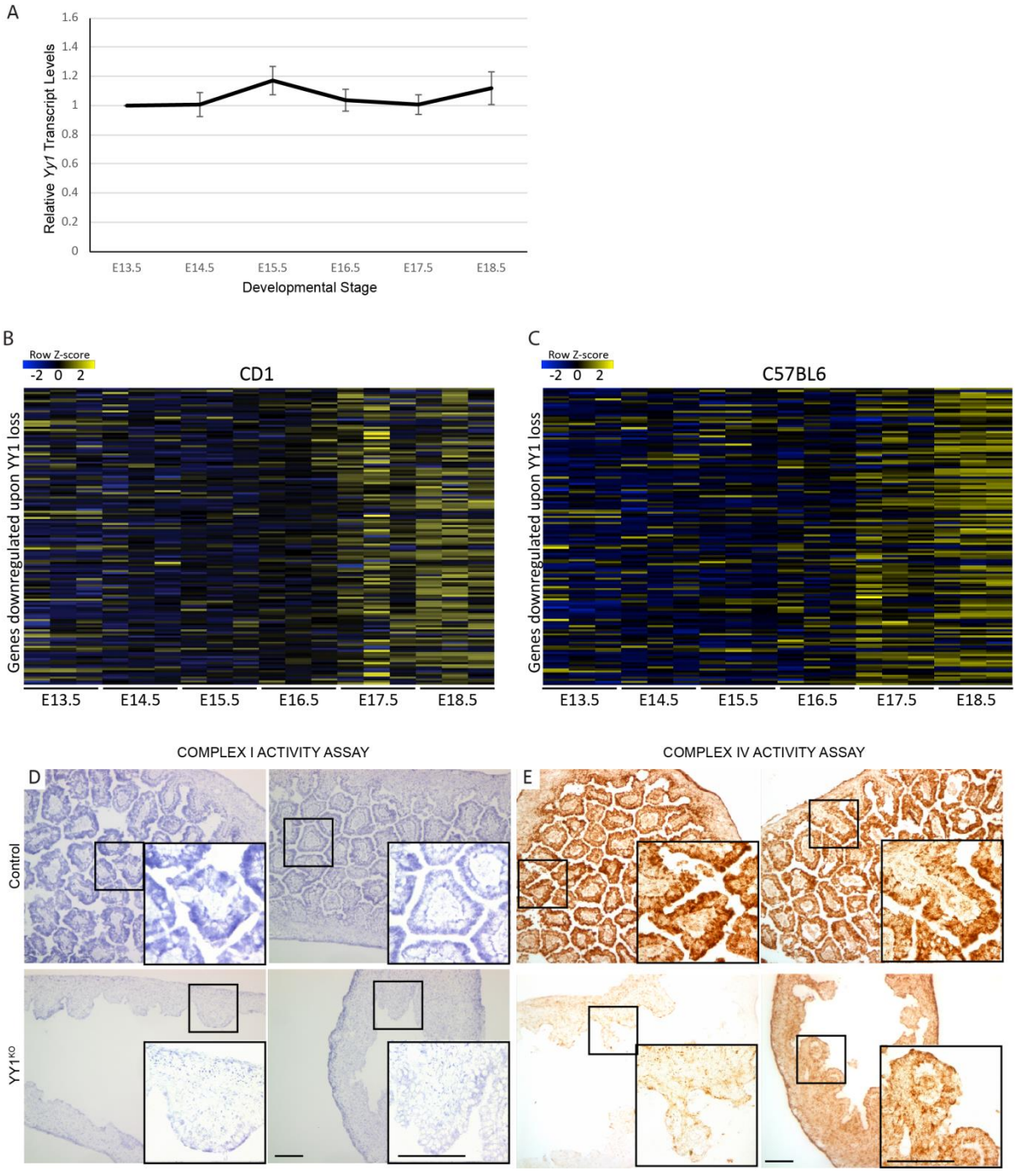
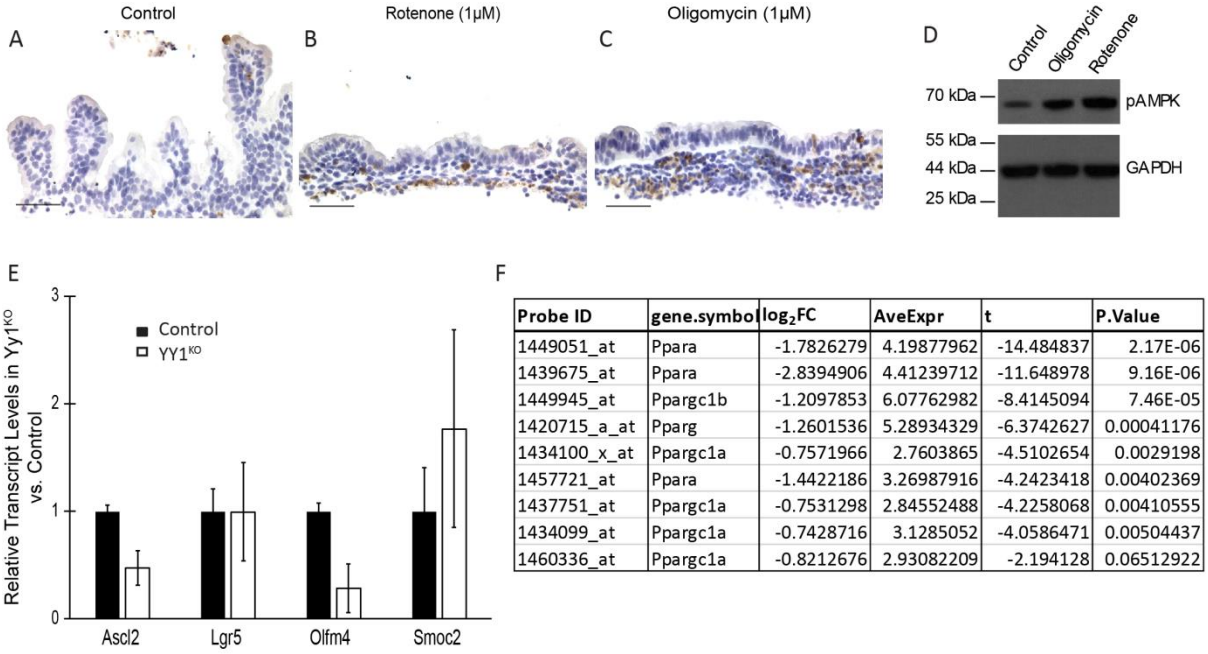


Fig. S4 **Genes downregulated upon YY1 loss are destined to increase expression, coincident with the onset of villus elongation.**
(A) Yy1 RNA levels don't change appreciably during murine intestinal development. Relative Yy1 levels were calculated based upon the average readings at 3 different microarray probes, across 12 replicate samples for each timepoint (GSE5204). Bars represent the SEM between the probes. Developmental time course showing expression of YY1 downregulated genes in mouse intestine in CD1 (B) and C57BL6 genetic backgrounds (C). Genes include those downregulated > $\log_2$ fold change > 1.5 and with p-value < 0.05 that are included in the developmental timecourse microarray data (GSE5204). (D-E) Electron transport chain complex activities are diminished in the YY1 mutant epithelium, 2 additional biological replicates to accompany those in figure 5D-E. scale = 50 µm.



F

Probe ID	gene.symbol	log_2FC	AveExpr	t	P.Value
1449051_at	Ppara	-1.7826279	4.19877962	-14.484837	2.17E-06
1439675_at	Ppara	-2.8394906	4.41239712	-11.648978	9.16E-06
1449945_at	Ppargc1b	-1.2097853	6.07762982	-8.4145094	7.46E-05
1420715_a_at	Pparg	-1.2601536	5.28934329	-6.3742627	0.00041176
1434100_x_at	Ppargc1a	-0.7571966	2.7603865	-4.5102654	0.0029198
1457721_at	Ppara	-1.4422186	3.26987916	-4.2423418	0.00402369
1437751_at	Ppargc1a	-0.7531298	2.84552488	-4.2258068	0.00410555
1434099_at	Ppargc1a	-0.7428716	3.1285052	-4.0586471	0.00504437
1460336_at	Ppargc1a	-0.8212676	2.93082209	-2.194128	0.06512922

Fig S5. **Mitochondrial inhibitors restrict villus growth, but do not increase cell death.** (A-C) cleaved caspase 3 immunostaining on tissue explants from the indicated treatments. (D) pAMPK levels were elevated upon treatment with mitochondrial inhibitors, confirming expected activity of these compounds in compromising electron transport chain function and reducing cellular ATP levels. (E) Transcript levels of markers of the Lgr5+, crypt base columnar cell population were assayed in control versus Yy1 knockout E18.5 intestinal epithelium. Data were normalized to actin transcript levels, n=3 control and 5 knockout; bars indicate standard error. (F) Regulatory changes in PPAR and PPARGC1 family members upon Yy1 loss in E15.5 epithelium, detected by microarray, showing that several of these known mitochondrial regulators are diminished upon Yy1 loss.

Table S1. The table includes three tabs reporting (1) differentially expressed genes in the *Yy1* knockout E15.5 intestinal epithelium, (2) list of oxidative phosphorylation genes used in Fig. 7C, and (3) genes bound by YY1 in adult intestine ChIP-seq and downregulated in E15.5 intestinal epithelium.

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