170809 - Striatal SMAD3 Blot

SMAD3 regulation is implicated in Seth Ament / Jocelynn Pearl's HD transcription factor analysis paper. Requested Western blot evidence of dysregulation at the protein or phospho-protein level. To match with SMAD3 ChIP-seq, 4mo striata (and cortex, though they aren't used here) were collected from WT and $Htt^{Q111/+}$ mice, with protease and phosphatase inhibitors to enable blotting of phospho-SMAD3. This is replicating the Aug. 2, 2017 blot, but we got a new antibody in from Abcam to assess pSMAD3 levels. Lysate has been through one freeze-thaw cycle.

SMAD3 validation plan is here, and protein quants are here and here. Protein preparation and loading notes are here. Loaded 50ug per lane.

Aug. 9, 2017

Gel Layout

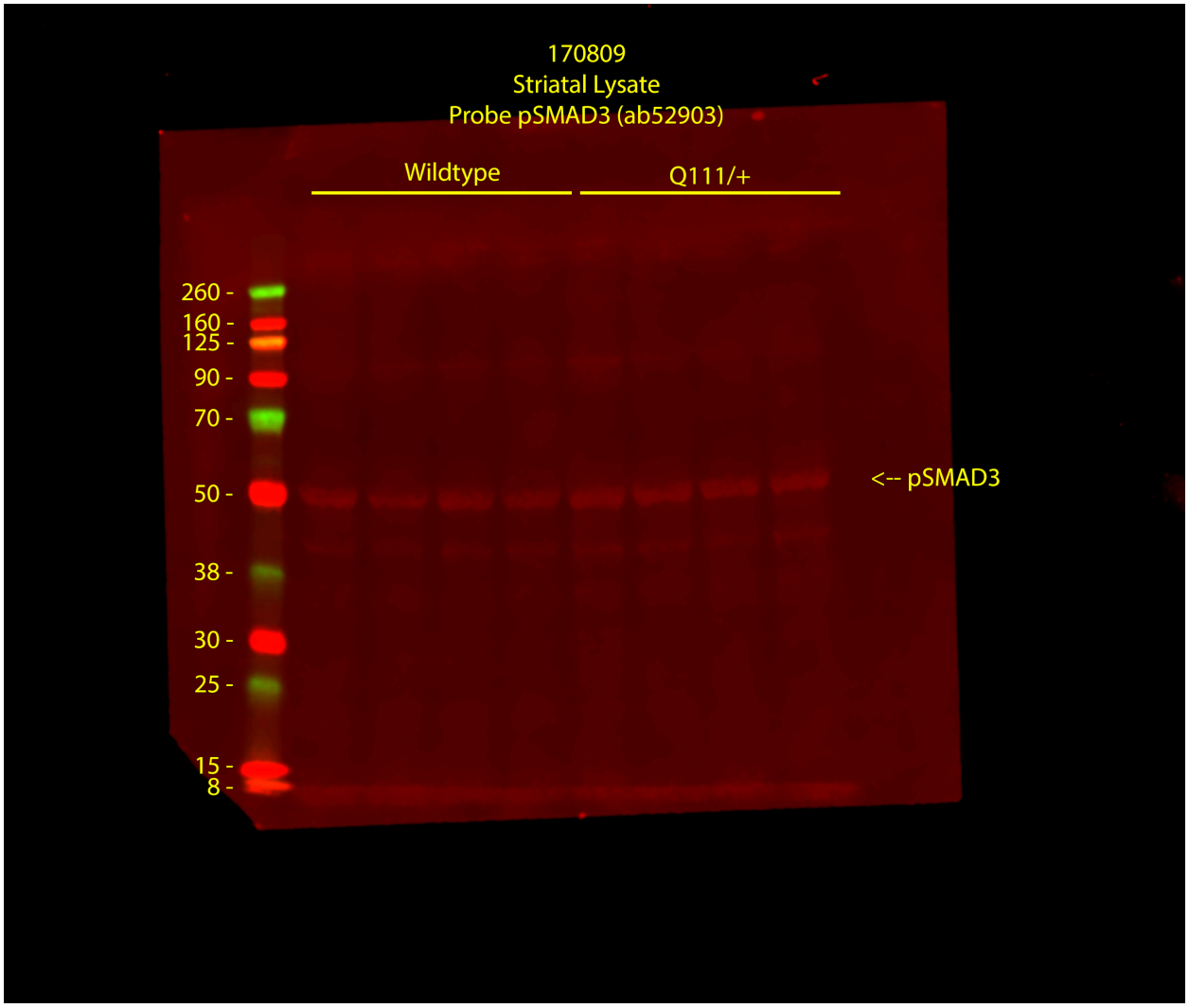
Lane 1:	12uL	Chameleon Duo ladder (Li-Cor)		
Lane 2:	50ug	TZ1	Striatum	WT
Lane 3:	50ug	TZ2	Striatum	WT
Lane 4:	50ug	TZ3	Striatum	WT
Lane 5:	50ug	TZ4	Striatum	WT
Lane 6:	50ug	XK1	Striatum	$Htt^{Q111/+}$
Lane 7:	50ug	XL1	Striatum	$Htt^{Q111/+}$
Lane 8:	50ug	XS2	Striatum	$Htt^{Q111/+}$
Lane 9:	50ug	XS3	Striatum	$Htt^{Q111/+}$
Lane 10:	EMPTY			

- Ran in 10% bis-tris gel 1hr at 200V with MOPS running buffer
- Blotted at 30V for 90min on ice
- Blocked 1hr at RT in LiCOr Odyssey blocking buffer (TBS based).
- Incubated O/N at 4C in Abcam rabbit anti-phospho SMAD3 ab52903 (1:1000) diluted in Odyssey TBS blocking buffer

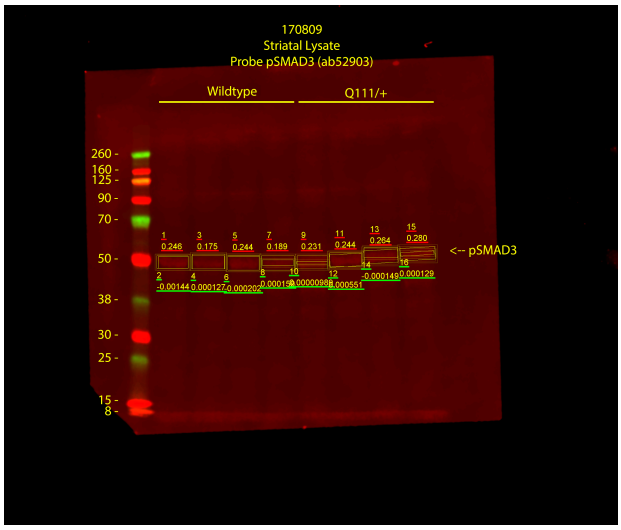
Aug. 10, 2017

- Washed 3x5min in TBS-Tw
- Incubate 45min in Li-Cor anti-Rb secondary (1:10,000 in Odyssey TBS blocking buffer + 0.05% Tween + 0.01% SDS)
- Washed 3x5min TBS-Tw followed by 2x10min PBS
- Imaged on Li-Cor using 700 and 800 settings, 800 only for the ladder to make the green bands fluoresce

Blot 170809 - Probe pSMAD3 ab52903



And quantitated, in case something goes terribly wrong.

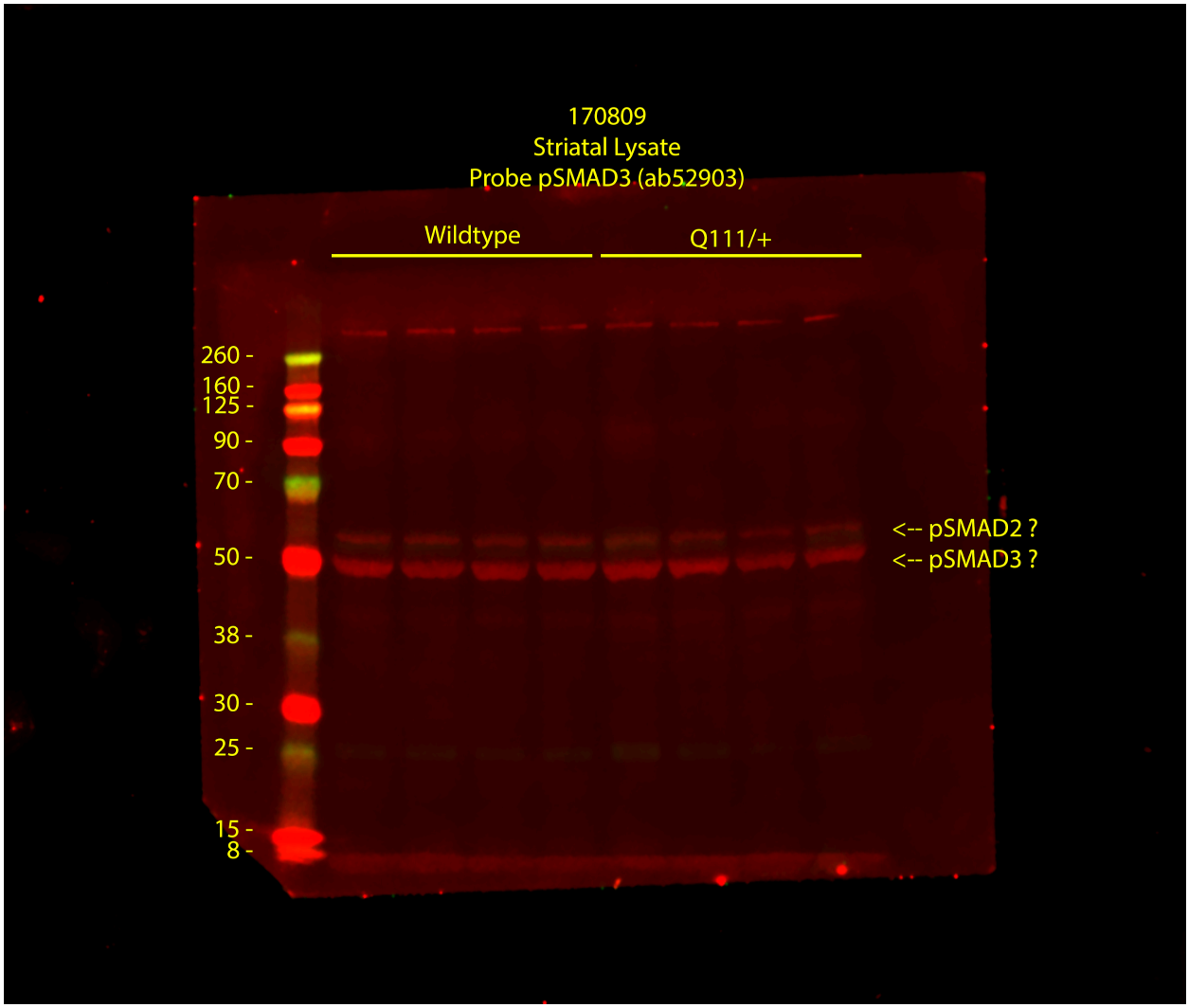


As you can see, the pSMAD3 signal is pretty weak, so it's back in ab52903 (at 1:500 now) overnight. I'll pull it tomorrow and probe again. Weakness aside, it's the right size. By eye, it's about the same between Q111 and WT, though I'll wait to make a call until after re-doing the ab52903 and the ActB are done.

Aug. 11, 2017

- Washed 3x10min in TBS-Tw
- Incubate 1hr in fresh Li-Cor anti-Rb secondary (1:10,000 in Odyssey TBS blocking buffer + 0.05% Tween + 0.01% SDS)
- Washed 3x5min TBS-Tw followed by 2x5min TBS
- Imaged on Li-Cor using 700 and 800 settings, 800 only for the ladder to make the green bands fluoresce

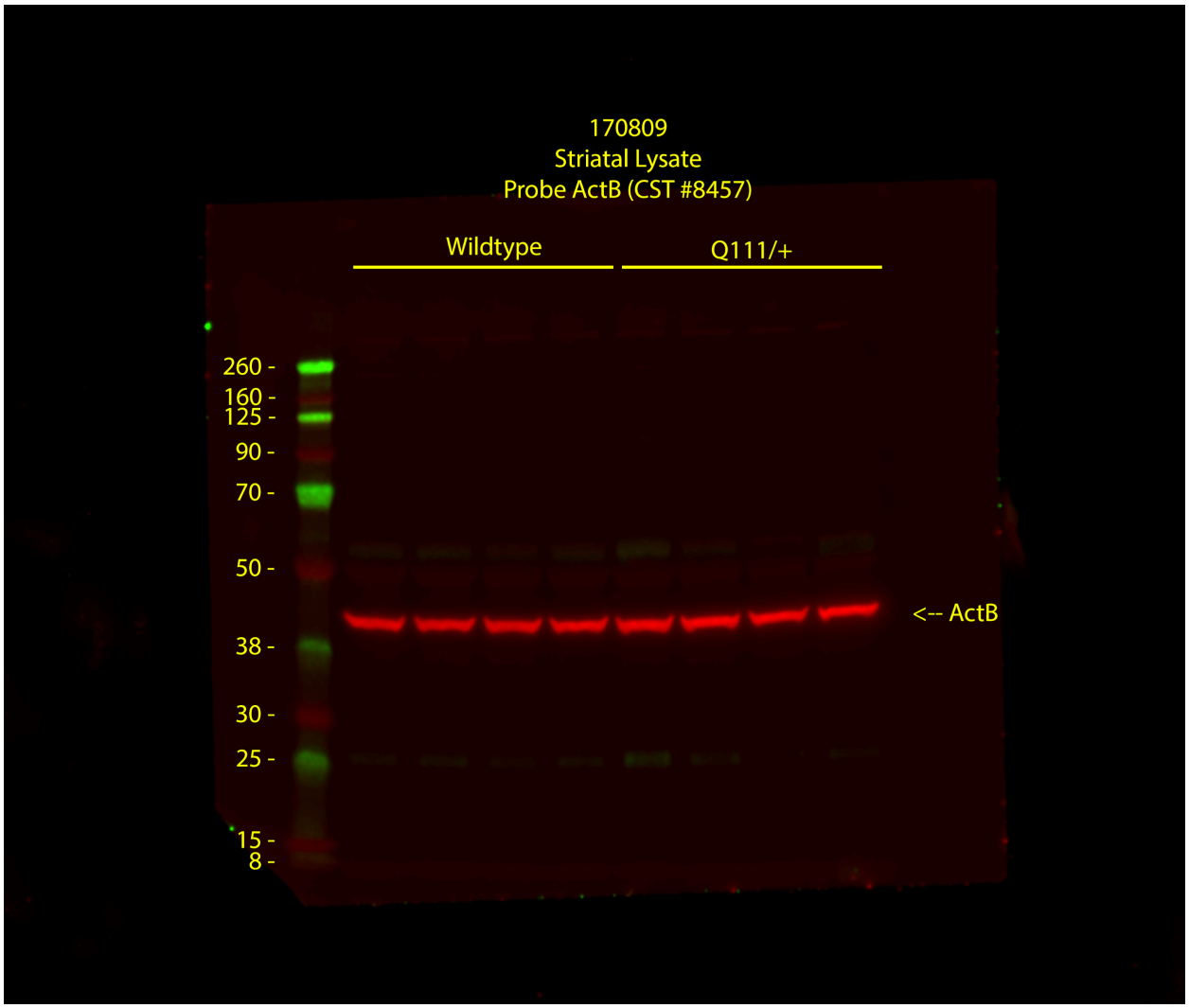
Blot 170809 - Probe pSMAD3 ab52903 (1:500)



Hm. I'm thinking the lower band is the SMAD3 and the upper band is SMAD2 showing up again. They have pretty high homology in the epitope, so that wouldn't be too unusual.

- Incubate 2hr at RT in ActB (CST #8457, 1:1000 in Odyssey block + 0.05% Tw) shaking at RT
- Wash 3x5min TBS-Tw
- Incubate 45min in new Li-Cor anti-Rb secondary (1:10,000 in Odyssey TBS blocking buffer + 0.05% Tween + 0.01% SDS)
- Washed 3x5min TBS-Tw followed by 2x10min TBS
- Imaged on Li-Cor using 700 and 800 settings, 800 only for the ladder to make the green bands fluoresce

Blot 170809 - Probe ActB (with some pSMAD3 signal still visible)



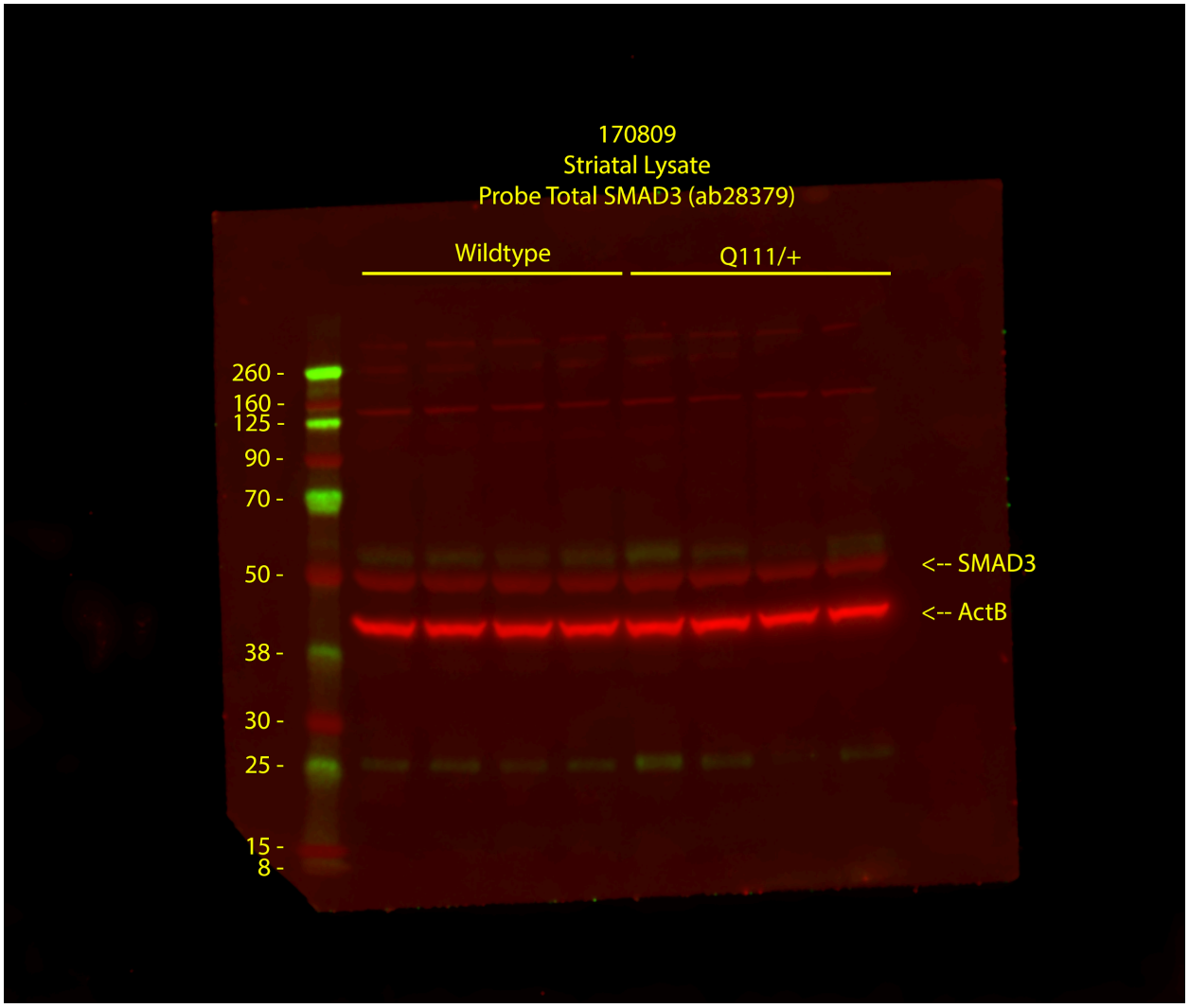
Red channel exposure much lower than for pSMAD3. ActB is super bright. Looks good.
Re-probe for total SMAD3 and quantitate the ActB.

- Incubate O/W in anti-total SMAD3 (Abcam catalog ab28379; 1:1000 made in Odyssey block + 0.05% Tween) at 4C

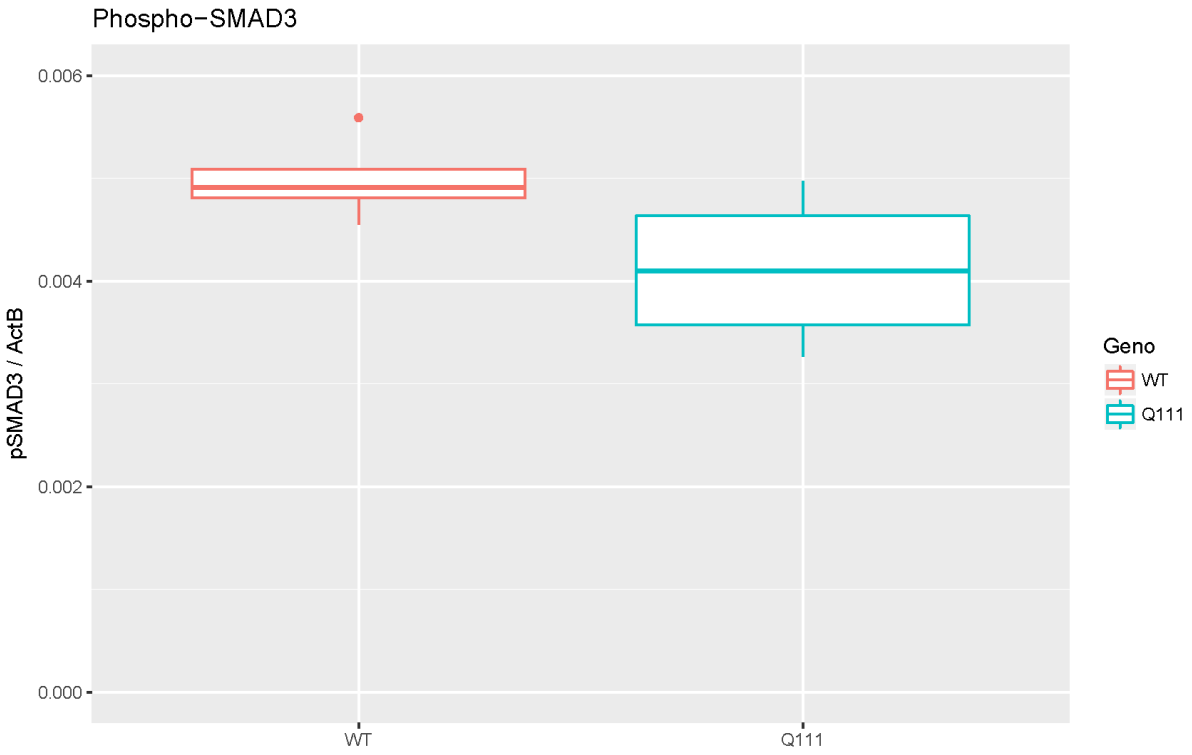
Aug. 14, 2017

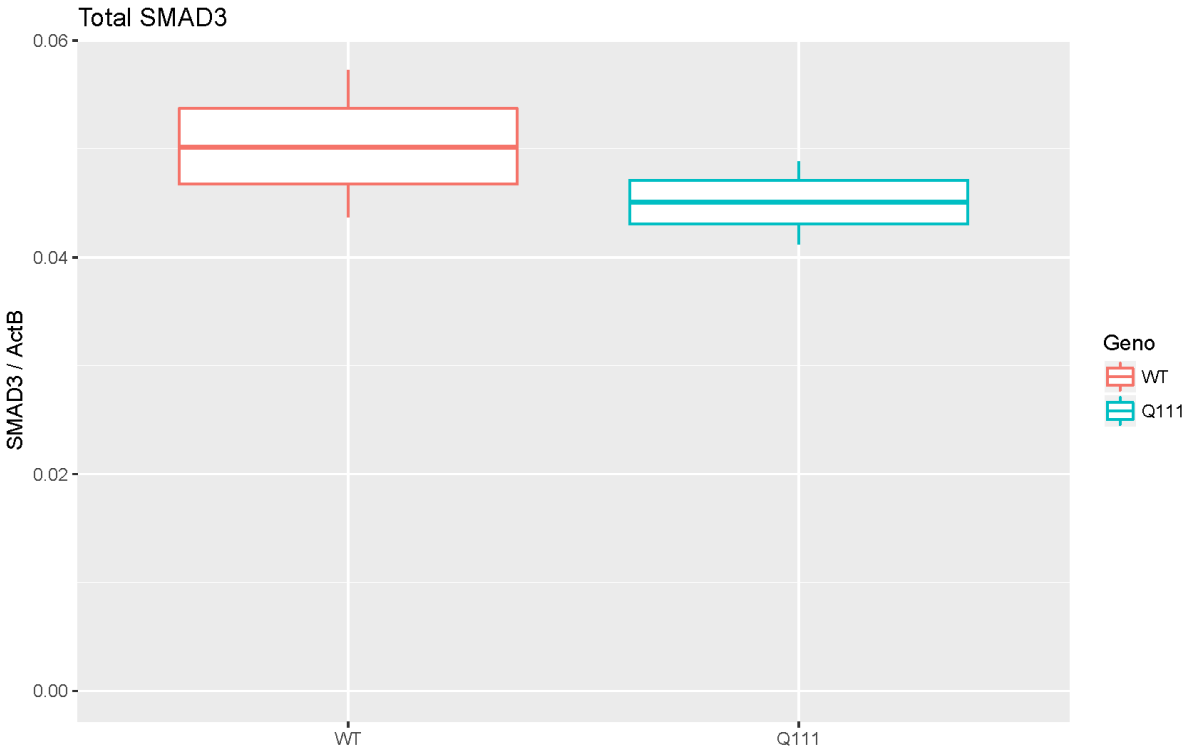
- Wash 3x5min TBS-Tw
- Incubate 45min in new Li-Cor anti-Rb secondary (1:10,000 in Odyssey TBS blocking buffer + 0.05% Tween + 0.01% SDS)
- Washed 3x5min TBS-Tw followed by 2x10min TBS
- Imaged on Li-Cor using 700 and 800 settings, 800 only for the ladder to make the green bands fluoresce

Blot 170809 - Probe Total SMAD3 ab28379 (with ActB signal still visible)



Where’s that mouse (green) signal coming from? Regardless, let's quant this and see how it looks.



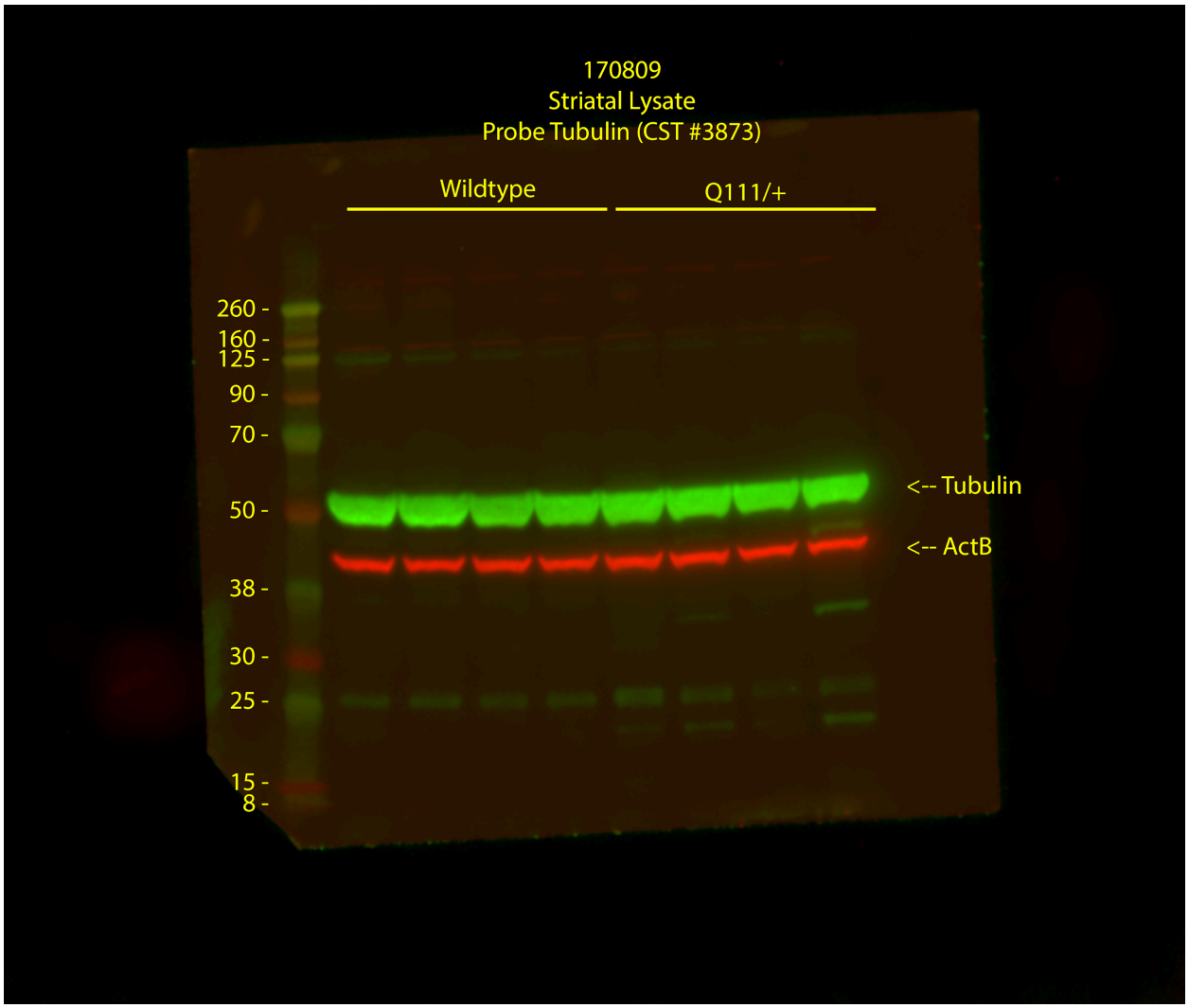


Aug. 24, 2017

Have new batch of tubulin antibody raised in mouse. Use this blot and 170817 blot as a test.

- Incubate 90 minutes in anti-tubulin (CST catalog #3873; 1:1000 made in Odyssey block + 0.05% Tween) at RT
- Wash 3x5min TBS-Tw
- Incubate 1hr in Li-Cor anti-Rb secondary (1:10,000 in Odyssey TBS blocking buffer + 0.05% Tween + 0.01% SDS)
- Wash 2x5min TBS-Tw, 2x5min TBS

Blot 170809 - Probe Tubulin CST #3873 (with ActB signal still visible)



Tubulin looks bright! Can take it down to an hour at RT. Definitely some non-specific bands as well, should back off on concentration, try (1:3000) or (1:5000).