Table S1. The relative intra-embryonic concentrations of caged Rockout (cRO) and decaged Rockout (RO) as determined by liquid chromatography/mass spectrometry (LC/MS)

Exposure	Stage	RO (nM)	cRO (nM)
Media alone	38	NF	<100
Solvent (–UV)	38	<10	<120
Solvent (+UV)	38	<10	<100
15 μM cRO (–UV)	38	16[¶]	45173*,[#]
15 μM cRO (+UV)[§]	38	275[‡,¶]	5357[#]
Media alone	45	NF	<100
Solvent (–UV)	45	<10	<100
Solvent (+UV)	45	<10	<250
15 μM cRO (–UV)	45	94**	11965*,[‡‡]
15 μM cRO (+UV)[§]	45	1321[‡,**]	1068[‡‡]

Tailbud stage embryos (stage 37) were cultured in medium plus the indicated compounds for 2 hours, rinsed and then kept in the dark or subjected to 1 minute 365 nm decaging (+UV). For each condition, whole embryos were harvested immediately after uptake and decaging (stage 38) or were cultured in media alone and guts were dissected at the completion of gut morphogenesis (stage 45). For LC/MS, excess medium was removed and the tissues from ten embryos were pooled and dissolved in acetonitrile with three cycles of freeze-thaw plus vortex agitation. The acetonitrile extracts were then spun at full speed in a microcentrifuge at 4°C for 5 minutes. The clear supernatant was analyzed by LC/MS at the NCSU Genomic Sciences Laboratory.

*The lower concentration of cRO at stage 45 versus stage 38 reflects its gradual diffusion out of the embryo during culture in media alone.

[‡]The higher concentration of RO observed at stage 45 versus stage 38 reflects its enrichment in the dissected gut tissues.

[§]+UV samples were irradiated on only the right-hand side of the prospective gut tube; thus, cRO is expected to be only partially decaged.

[¶]Concentration of RO in +UV sample is 17-fold higher than in –UV.

[#]Concentration of cRO in +UV sample is decreased by 88% compared with –UV.

**Concentration of RO in +UV sample is 14-fold higher than in –UV.

[‡‡]Concentration of cRO in +UV sample is decreased by 91% compared with –UV.

NF, not found.