Supplementary Materials for

Signaling dynamics control cell fate in the early Drosophila embryo

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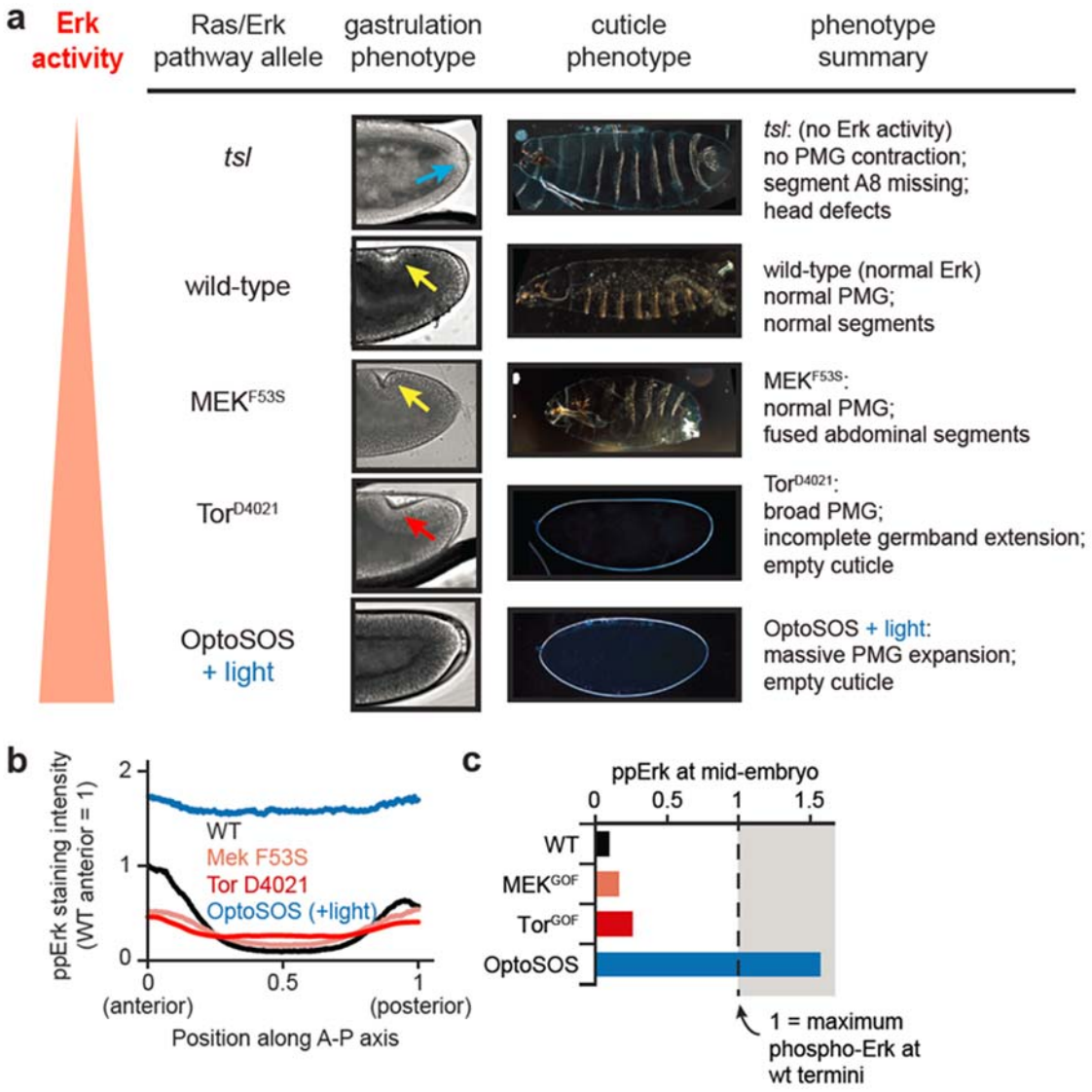

Figure S1: Related to Figure 1. Erk activity generated by various gain-of-function Erk pathway mutants. (a) Gastrulation and cuticle phenotypes of an allelic series of increasing Erk activation, from a loss-of-function allel (*tsl*) through wild-type, to two gain-of-function alleles (MEK and Tor), and finally optogenetic activation. Severity of Erk gain-of-function phenotypes increases through this series (right column). **(b)** Phospho-Erk (ppErk) levels were quantified in wild-type embryos and each gain-of-function background as a function of anterior-to-posterior position across individual embryos stained for ppErk. ppErk staining data was compiled and analyzed from previously-published raw images (Johnson, et al., 2017; Goyal, et al., 2017; Grimm, et al., 2012) by normalizing mutant ppErk staining to wild-type embryos that were stained in each of those experiments. (**c**) The average Erk level was quantified in the middle-most 20% of the embryo for the plot shown in **b**. The mid-embryo was chosen because Erk is not normally active there, so any Erk activity is a consequence of gain-of-function pathway activity.

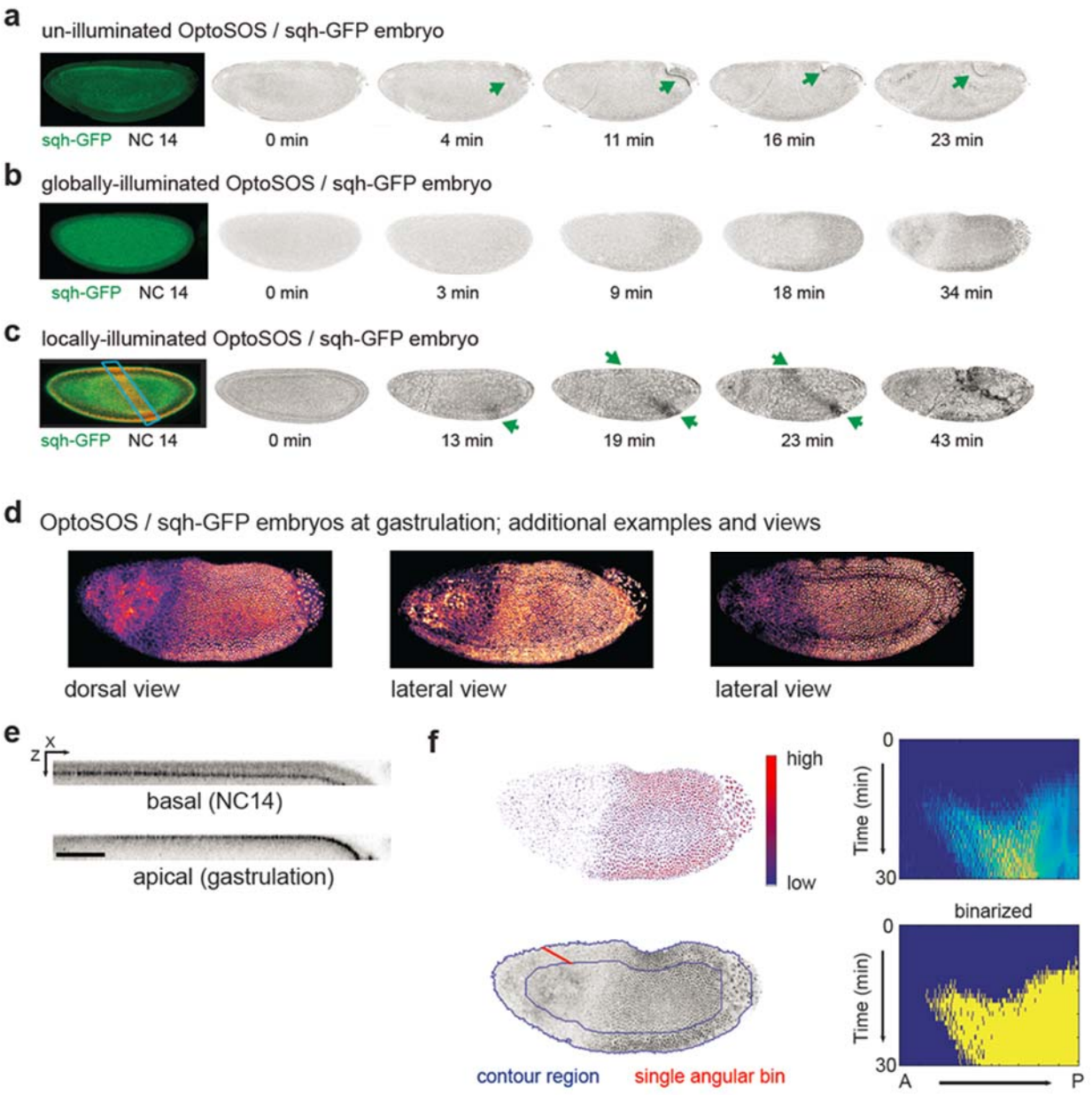

Figure S2: Related to Figure 1. Characterization of apical myosin in illuminated OptoSOS embryos. Montage of OptoSOS embryos from the start of gastrulation with (**a**) minimal photoactivation (Movie S1), (**b**) maximal, global blue light exposure (Movie S2), (**c**) or local activation (Movie S3). Arrows point to frames in which apical myosin localization is first seen at various embryonic positions. (**d**) Additional sqh-GFP image examples of optogenetically-activated embryos at various orientations before and during gastrulation. (**e**) Axial projections of Sqh-GFP at NC14 and gastrulation in the illuminated OptoSOS embryo from Movie S2; scale bar: 50 μm. (**f**) Analysis of myosin localization. Top left: apical myosin after background removal. Bottom left: Mask of contour region in which myosin intensity is calculated (blue) and an example of a single angular bin within it (red). Right: "Map" of myosin intensity (warm colors indicate more apical myosin) as a function of angular position from anterior to posterior and time (top). This map is then binarized for the purpose of calculating myosin timing (bottom).

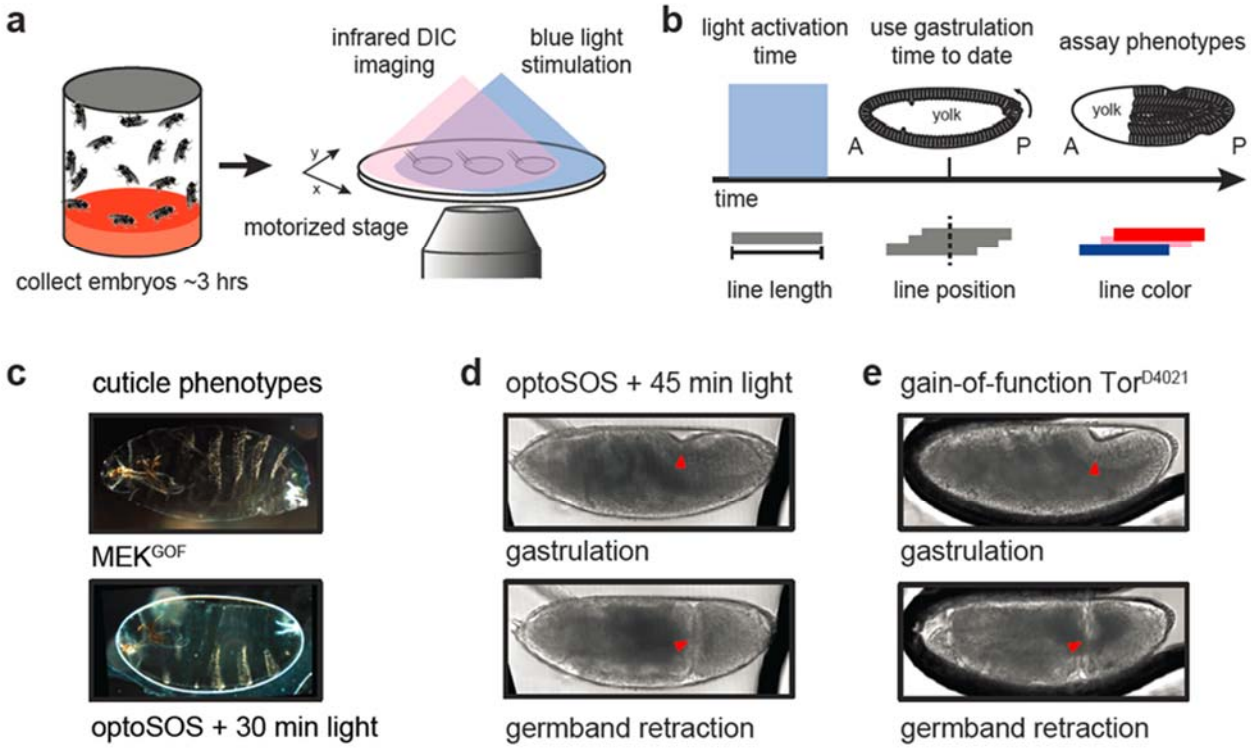

Figure S3: Related to Figure 3. Characterization of the duration-dependent fate switch. (a) Schematic of the experiment setup for the data shown in Figure 3C. Embryos are collected over a broad range of time and mounted and imaged under infrared light. During this time they are stimulated for a set duration with a maximally activating intensity of blue light. The time of gastrulation is then determined from the movies relative to the stimulation time. (**b**) A line is then drawn to represent the time and duration of each embryo, color-coded based on its eventual fate. (**c**) Representative examples of cuticles for a maternally driven MEK^{F53S} (bottom) and 30 minutes of optogenetic stimulation (top). (**d**) Comparison of the gastrulation phenotypes of 45 minutes of optogenetic stimulation with (**e**) embryos from $Torso^{D4021}$/+ mothers. During gastrulation (top) an abnormally large PMG invagination is formed, and germband extension stops prematurely. This causes the posterior end of the embryo to pinch off later at the time of germband retraction (bottom).

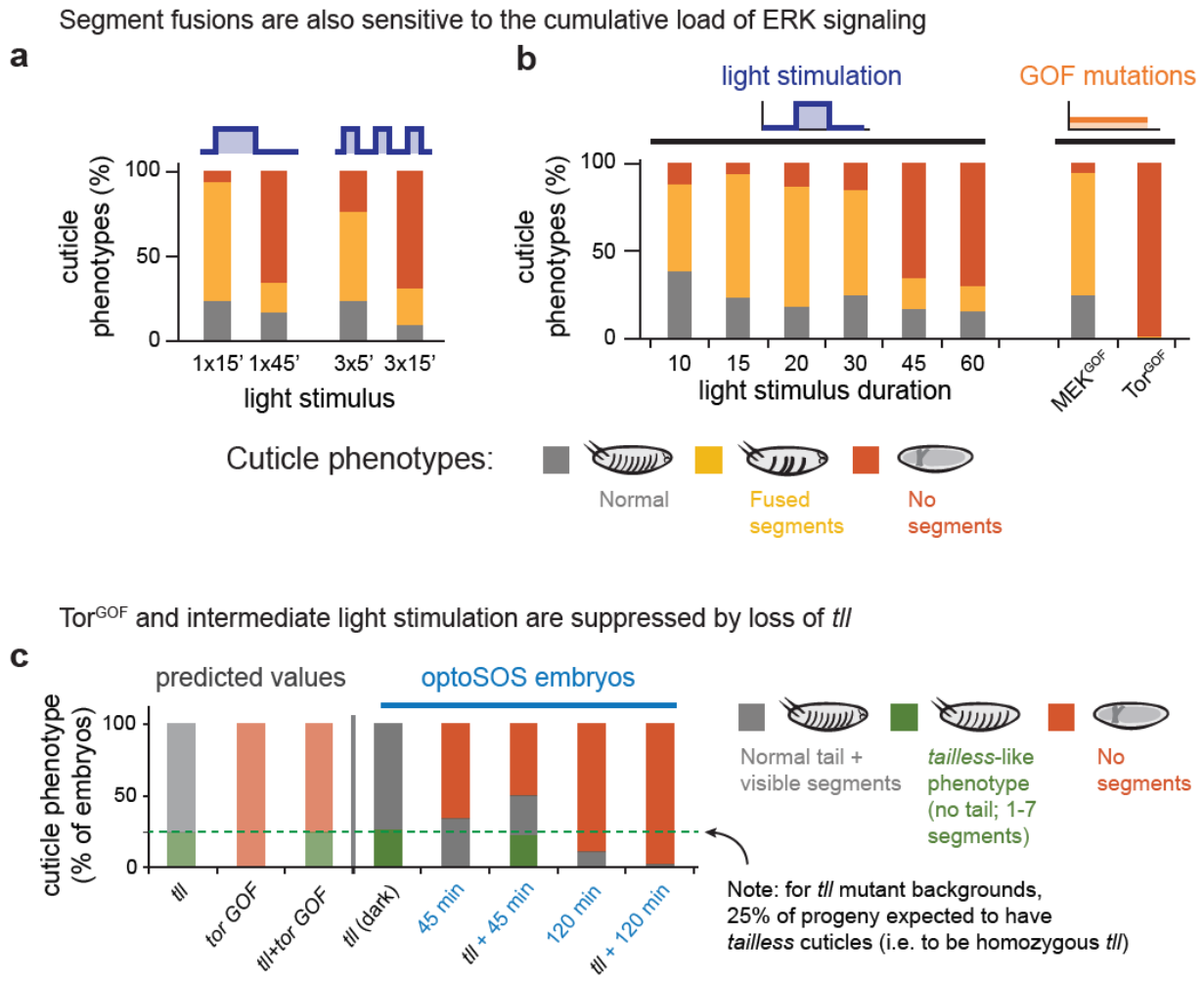

Figure S4: Related to Figure 4. Erk duration and mutant effects on cuticle phenotypes. (a-b) Segment fusions measured by cuticle preparation are also sensitive to the cumulative load of ERK signaling. **(a)** Cuticle phenotypes for optoSOS embryos stimulated with various pulses of light, similar to as in Figure 4A. Segment fusions similar to Figure S3C are observed after a single 15 min pulse or three 5 min pulses of light. **(b)** Cuticle phenotypes for various durations of optogenetic stimulation between 90-135 min post fertilization, compared to those obtained from Erk pathway GOF mutants. (MEK^{GOF}: MEK F53S; Tor^{GOF}: TorD4021). (**c**) Rescue of ectopic Erk activity by loss of *tll*. Twenty-five percent of embryos with *tll* backgrounds will be zygotic deficient for *tll*, thus lacking filzkörper (FK) and one or more abdominal segments. Gain-of-function torso mutations, as well long optogenetic Erk activations of 45 minutes or more result in a failure of gastrulation and thus the lack of formation of any denticle belts. Removal of *tll* is known to repress Torso GOF mutations and also represses the phenotype of 45 minutes of optogenetic activation (for which the phenotype resembles the Tor^{D4021}), thus giving the *tll* cuticle phenotypes for the 25% of embryos which are homozygous for the mutation. However loss of *tll* does not repress the long optogenetic activation of Erk, which also gives a distinct global contraction phenotype. The data on the left of the dashed line represent the hypothetical expected values for these mutations and the values are shown for various optogenetic combinations on the right.

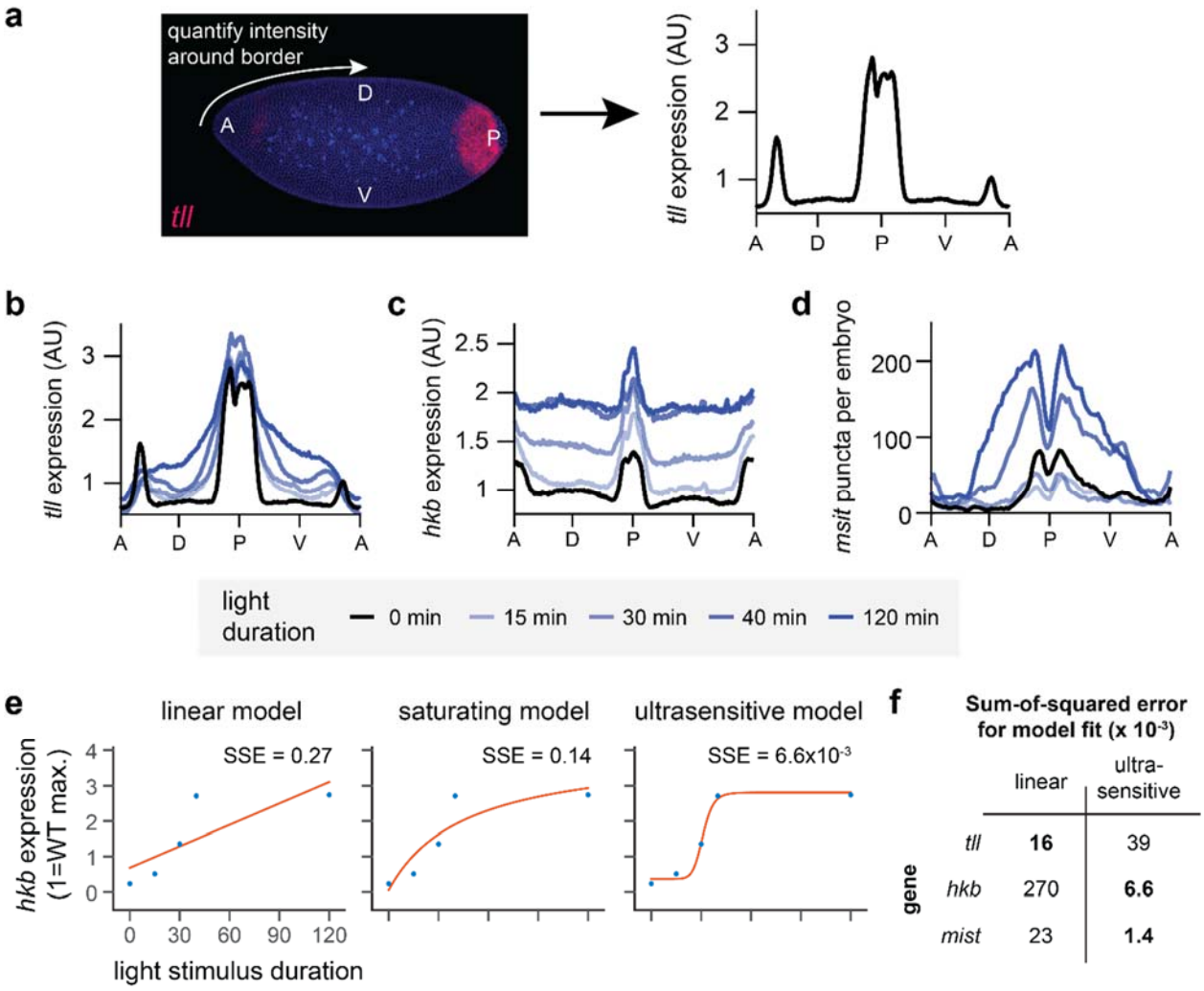

Figure S5: Related to Figure 5. Erk duration and mutant effects on cuticle phenotypes. (a) Schematic of how gene expression plots are generated. Intensity is is calculated along contours around embryo clockwise starting from the anterior pole. (**b-d)** Quantification of normalized gene expression levels across the embryo for (**b**) *tll*, (**c**) *hkb*, and (**d**) *mist.* **(e)** Comparison of the fit of various models for the *hkb* curve shown in Figure 5C and (**f**) the sum- of squared error for each model for all the curves in Figure 5B & 5C.