A

		Predicted	
		Yes	No
Actual	Yes	True positives (TP)	False negatives (FN)
	No	False positives (FP)	True negatives

$$\text{True Positive Rate } (TPR) = \frac{TP}{TP+FN}$$

$$\text{Positive Predictive Value } (PPV) = \frac{TP}{TP+FP}$$

$$\text{Error} = 1 - TPR \times PPV$$

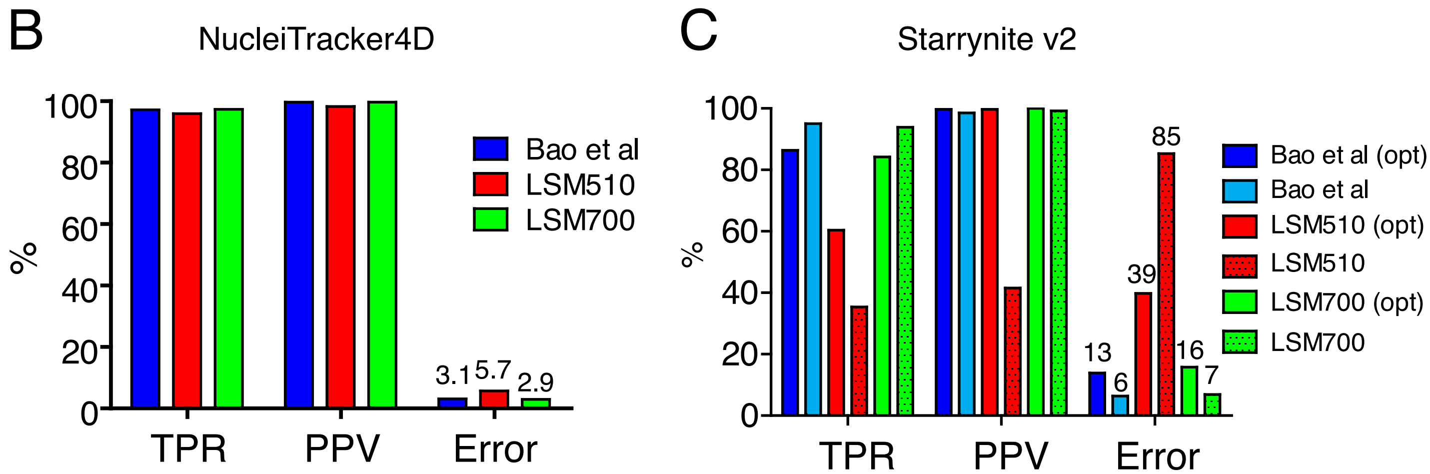

Fig. S1. Error rates in NucleiTracker 4D and other automated lineage software. (**A**) The confusion matrix and error metrics. (**B**) NucleiTracker4D performance. We used NucleiTracker4D to track nuclei over 99 to 115 minutes from the four-cell stage in three confocal data sets: the original data set from Bao et al. (Bao et al., 2006) (blue); one Zeiss LSM 510 movie (red); one dataset obtained from Zeiss LSM 700 (green) using NucleiTracker4D. NucleiTracker4D displays both a high true positive rate (TPR) and positive predicted value (PPV) across all data sets, resulting in very low cumulative error. (**C**) Starrynite v2 (Santella et al., 2010) performance. We first optimized the program parameters for each data set using curated data for the first 50 minutes (opt) and then ran the program with these optimized parameters up to 99 minutes.

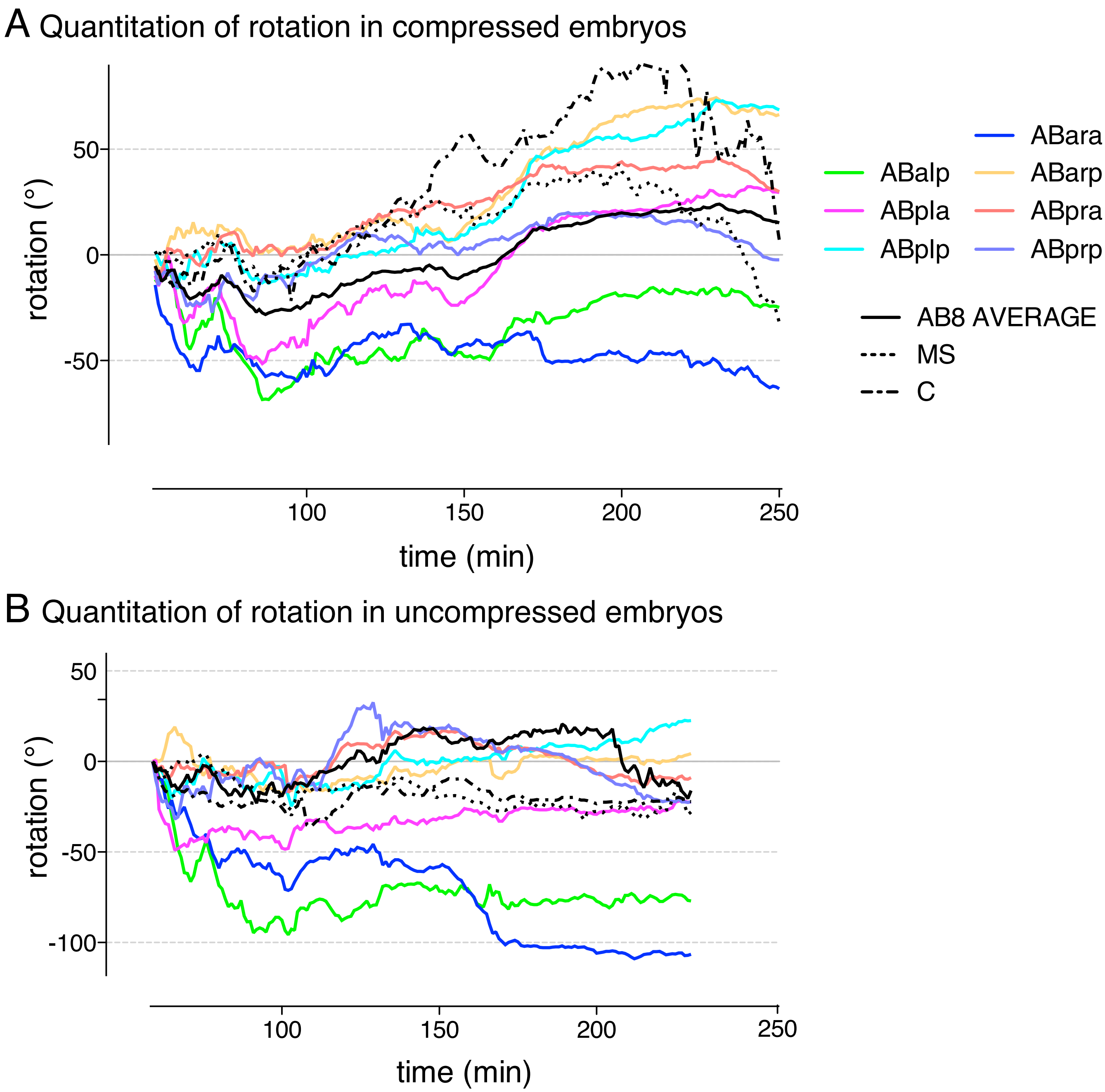

Fig. S2. The effect of compression on embryonic rotational movement and nuclear displacement. (**A**) The relative rotation of a given precursor and its descendants, expressed as degrees around the long axis of the embryo as viewed from the anterior pole. Angle of the precursor at 52 minutes is set as 0°. The angle of the center of gravity of the descendants is plotted over time, as is the average of the AB^8 groups (excluding ABala, which lies at the anterior pole and movements of which are not well captured by this metric). Note the clockwise rotation of most cell groups except ABalp and ABara between 150 and 180 minutes. In general, posterior AB cells rotate more than anterior AB cells. AB^8, MS and C color codes as in Movie 4. (**B**) Analysis of embryonic rotations in uncompressed embryos. Unlike confocal imaging, Bessel imaging does not constrain the embryo to a stereotypical orientation. We therefore rotated and aligned Bessel data sets to match the long axes and left-right symmetry of confocal imaged data sets, prior to analysis of embryonic rotation.

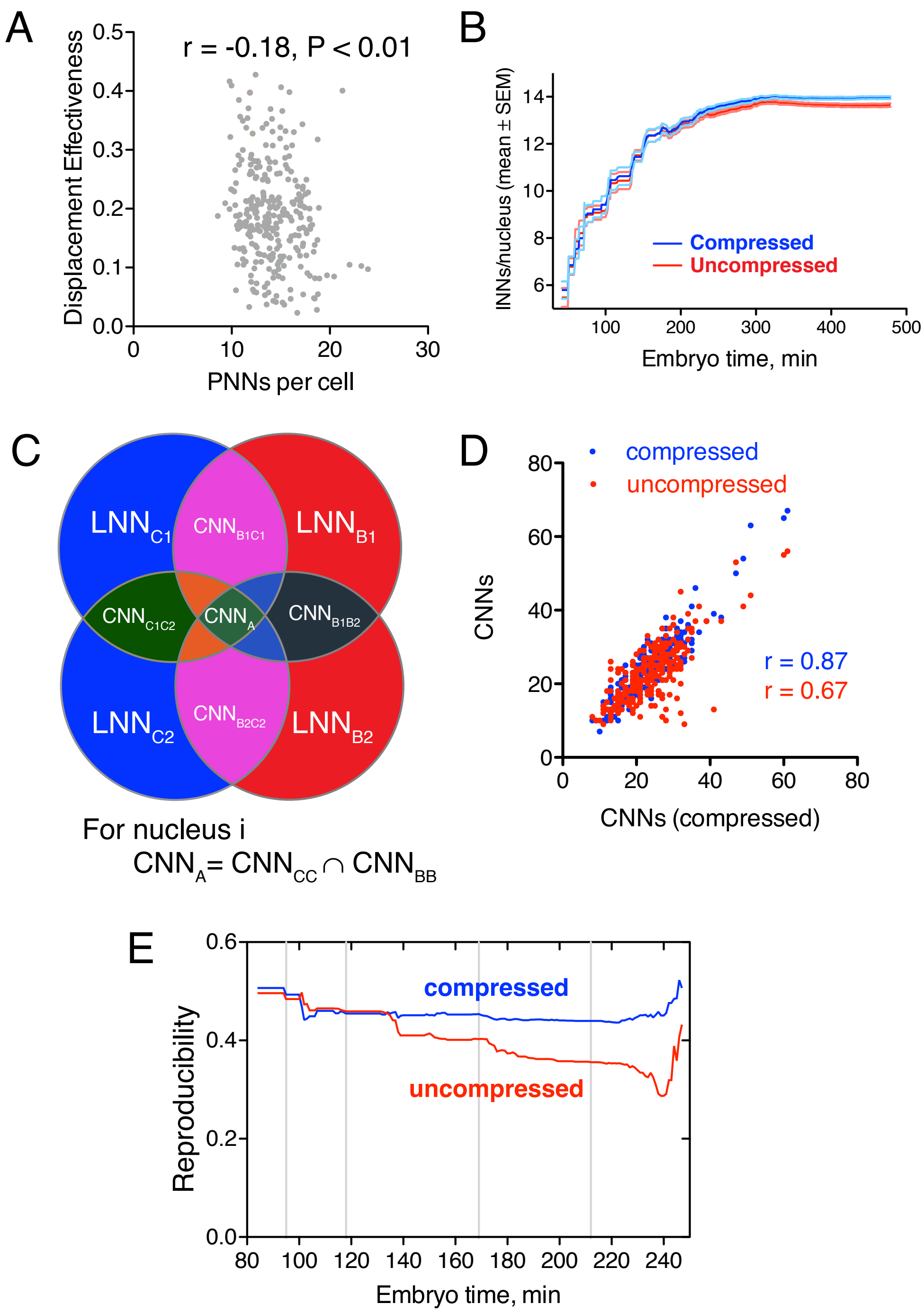

Fig S3. Nuclear nearest neighbors (NNs) and the effect of compression. (**A**) Negative correlation of persistent NNs with displacement effectiveness in a single confocal-imaged embryo. The net displacement of a nucleus throughout its lifetime is the vector from its position at birth to its position prior to division. The total displacement is the sum of stepwise migrations made by a nucleus throughout its lifetime. The displacement effectiveness (DE) is defined as the ratio of net to total displacement. (**B**) Evolution of the global mean number of instantaneous nearest neighbors (INNs) through embryonic development for wild-type compressed (blue) and wild-type uncompressed (Bessel imaging, red) embryos. At each time point the number of nearest neighbors of all nuclei is averaged for the entire embryo. Error bars indicate s.e.m. across the nuclei present in the embryo at a particular time. Compressed embryos display slightly higher mean INNs at late time points; c.f. Figure 5C of Hench et al. (Hench et al., 2009). (**C**) Set description of common nearest neighbors for a specific nucleus. The set of common nearest neighbors (CNNs) is defined as the LNNs for a specific nucleus consistently found in all data sets. A nucleus must be neighboring for at least a threshold minimum time to be counted as a NN. For each nucleus we make all possible pairwise comparisons among embryos imaged in confocal (C1, C2...*n*=3) or Bessel (B1, B2...*n*=3) conditions. (**D**) Typical correlations of CNN values (threshold=2 minutes) between individual compressed (blue) and uncompressed (red) embryos. Compressed embryos are more highly correlated with each other than are uncompressed embryos. (**E**) Uncompressed (Bessel) imaged embryos display lower embryo-embryo reproducibility in nearest neighborhoods compared with confocal imaged embryos. Reproducibility is defined as the ratio (CNN/LNN), averaged over the whole embryo at each time point.

Table S1. Shorthand names for ventral neuroblasts from ~220 to 280 minutes

E, D right	C right	B right	A right	A left	B left	C left	E, D left
E0R ABarappapa RMDDR/x,SMBDR		**B-1R** ABprpapaaa SMDDR/AIYR			**B-1L** ABplpapaaa SMDDL/AIYL		**E0L** ABalpapapa RMDDL/x,SMBDL
E1R ABprpaaaap exc socket G1/DB3		**B0R** ABprpapaaap SIADR/SIBVR			**B0L** ABplpapaaap SIADL/SIBVL		E1L ABplpaaaap exc duct/DB1
E2R ABarappapp DB2/SMBVR	**C1R** ABprppaapa x/RIMR	**B1R** ABprpapapa excglR/AVKR	**A1R** ABprpappaa RIH/AVL	**A1L** ABplpappaa RMEV/exc	**B1L** ABplpapapa AVKL/excglL	**C1L** ABplppaapa x/RIML	**E2L** ABalpapapp X/SMBVL
	C2R ABprppaapp AIAR/DB7	**B2R** ABprpapapp SIAVR/DA8	**A2R** ABprpappap RIS/DB4	**A2L** ABplpappap x	**B2L** ABplpapapp SIAVL/DB5	**C2L** ABplppaapp AIAL/DB6	
D1R ABprppapaa SABVR, RIFR/DA1	**C3R** ABprppappa RIGR/DD2	**B3R** ABprppppaa PVPR/repVR	**A3R** ABprpapppa RIR/AVG	**A3L** ABplpapppa K/K'	**B3L** ABplppppaa PVPL/repVL	**C3L** ABplppappa RIGL/DD1	**D1L** ABplppapaa SABVL,RIFL/SABD
D2R ABprppapap DA3/DA5	**C4R** ABprpppaaa Y/DA7	**B4R** ABprppppap B/DVA	**A4R** ABprpapppp virl/virR	**A4L** ABplpapppp PVT/repD	**B4L** ABplppppap U/F	**C4L** ABplpppaaa DA9/DA6	**D2L** ABplppapap DA2/DA4
	C5R ABprppappp DD4/DD6	**B5R** ABprpppapa PHshR/hyp	**A5R** ABprpppppa bm/sph	**A5L** ABplpppppa mu intL/an dep	**B5L** ABplpppapa PHshL/hyp	**C5L** ABplppappp DD3/DD5	
	C6R ABprpppaap PVCR,LUAR/ PHAR	**(B6R)** ABprpppapp x	**A6R** ABprpppppp spike/hyp10	**A6L** ABplpppppp spike/hyp10	**(B6L)** ABplpppapp x	**C6L** ABplpppaap PVCL/LUAL/ PHAL	