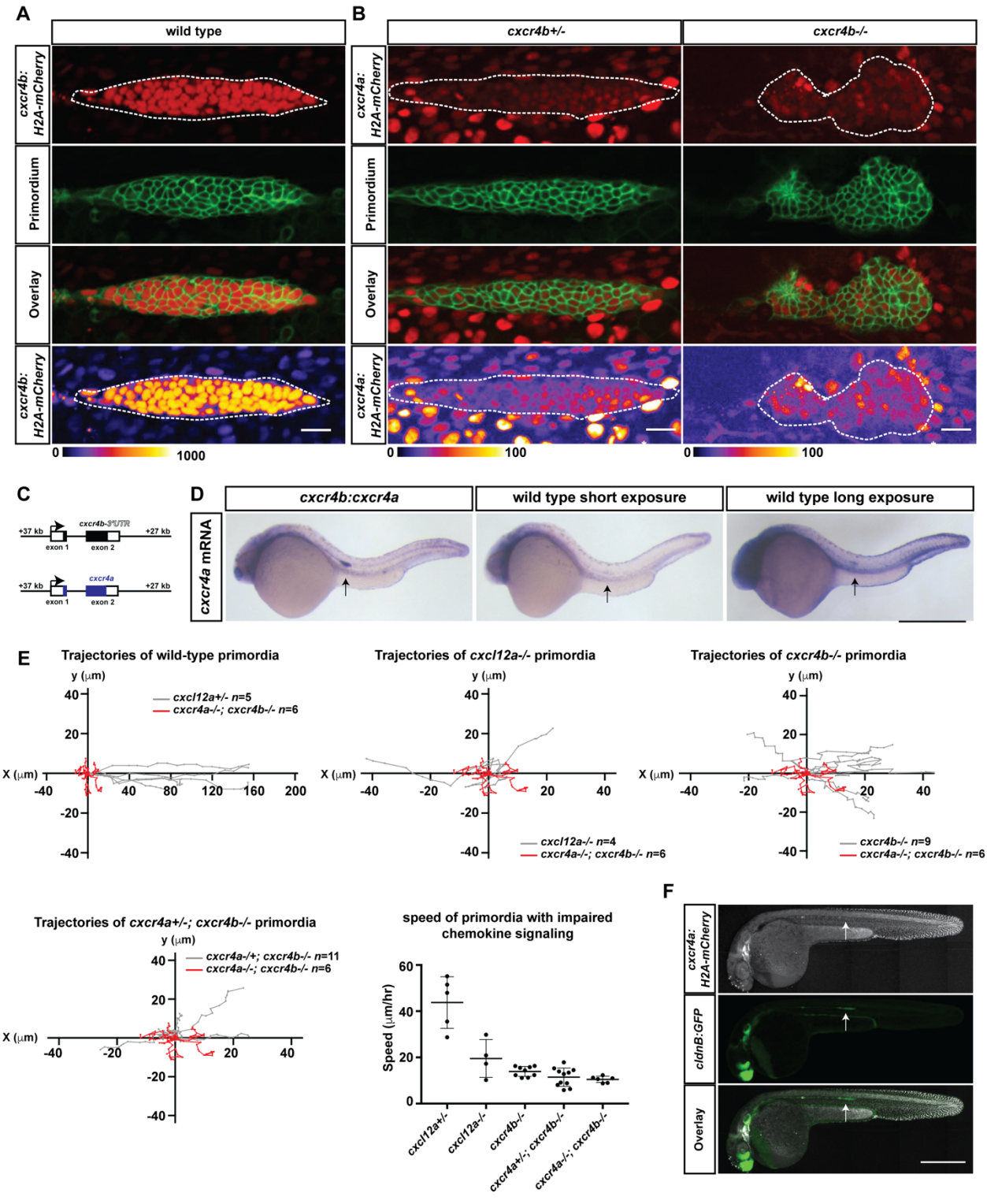

A
wild type
cxcr4b: H2A-mCherry
Primordium
Overlay
cxcr4b: H2A-mCherry
0
1000
B
cxcr4b+/-
cxcr4b-/-
cxcr4a: H2A-mCherry
Primordium
Overlay
cxcr4a: H2A-mCherry
0
100
0
100
C
+37 kb
cxcr4b-3'UTR
+27 kb
exon 1
exon 2
+37 kb
cxcr4a
+27 kb
exon 1
exon 2
D
cxcr4b:cxcr4a
wild type short exposure
wild type long exposure
cxcr4a mRNA
E
Trajectories of wild-type primordia
y (μm)
cxcl12a+/- n=5
cxcr4a-/-; cxcr4b-/- n=6
X (μm)
Trajectories of cxcl12a-/- primordia
cxcl12a-/- n=4
cxcr4a-/-; cxcr4b-/- n=6
Trajectories of cxcr4b-/- primordia
cxcr4b-/- n=9
cxcr4a-/-; cxcr4b-/- n=6
Trajectories of cxcr4a+/-; cxcr4b-/- primordia
cxcr4a-/+; cxcr4b-/- n=11
cxcr4a-/-; cxcr4b-/- n=6
speed of primordia with impaired chemokine signaling
Speed (μm/hr)
cxcl12a+/-
cxcl12a-/-
cxcr4b-/-
cxcr4a+/-; cxcr4b-/-
cxcr4a-/-; cxcr4b-/-
F
cxcr4a: H2A-mCherry
cldnB:GFP
Overlay

Figure S1. Expression pattern of Cxcl12 chemokine receptors and migration defects in Cxcl12 ligand and receptor mutants, Related to Figure 1

(A) Single confocal slice through primordium in live *cxcr4b:H2A-mCherry*; *cldnB:lyn$_2$GFP* embryo at 36 hpf. Lower panel shows H2A-mCherry intensities as fire map. Primordium is outlined with a dashed white line in top and bottom panels. Scale bar corresponds to 20 μm.

(B) Single confocal slices through primordia in live *cxcr4a:H2A-mCherry*; *cldnB:lyn$_2$GFP* embryos heterozygous (left panels) or mutant for *cxcr4b* (right panels) at 36 hpf. Lower panel shows H2A-mCherry intensities as fire map. Primordium is outlined with a dashed white line in top and bottom panels. Scale bar 20 μm.

Note, images shown in A and B were collected with identical laser powers and microscope settings.

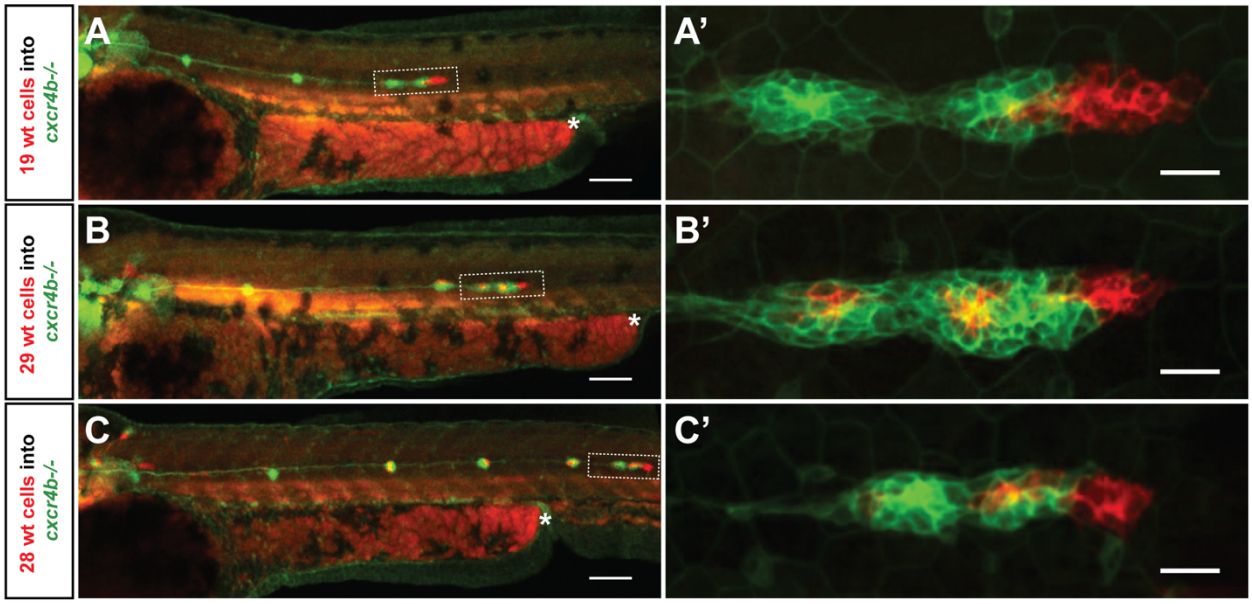
(C) Schematic of *cxcr4b* genomic locus (top) and *cxcr4b:cxcr4a* BAC transgene (bottom).

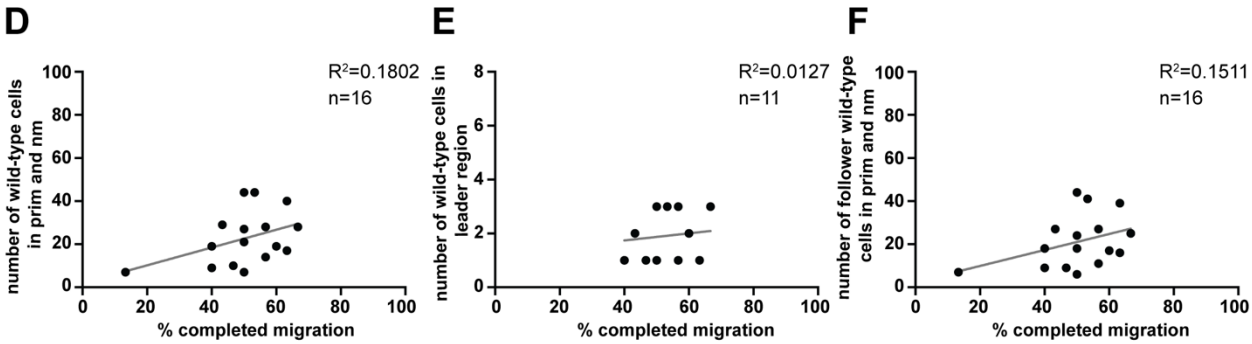
(D) *In situ* hybridization against *cxcr4a* mRNA in *cxcr4b:cxcr4a* (left) and wild-type (middle and right) embryos at 28 hpf. The wild-type embryo in the middle was stained for the same time as the *cxcr4b:cxcr4a* embryos. The wild-type embryo in the right panel was stained for a longer period to develop the staining for the endogenous *cxcr4a* mRNA more strongly. Arrows point to the primordium in each embryo. Scale bar corresponds to 0.5 mm.

(E) Trajectories and speed of primordia with reduction or loss of Cxcr4-mediated Cxcl12a signaling from time lapse videos. As a reference each graph shows the trajectories of *cxcr4a*; *cxcr4b* double mutant primordia in red. Genotypes and n are indicated.

(F) Tiled and sum projected Z-stacks of whole embryo transgenic for *cxcr4a:H2A-mCherry*; *cldnB:lyn$_2$GFP* at 36 hpf. Scale bar corresponds to 0.5 mm.

N indicates the number of chimeric primordia in D-F.





G

Overall wt cell number in *cxcr4a+/-; cxcr4b-/-* primordia	Wt leader cell number in *cxcr4a+/-; cxcr4b-/-* primordia	Wt follower cell number in *cxcr4a+/-; cxcr4b-/-* primordia	% completed migration
16	3	13	40
21	4	17	30
28	4	24	50
56	2	54	50
19	5	14	46.7
10	1	9	50
51	1	50	73.3
45	2	43	53.3
30	4	26	56.7
27	6	21	40
19	2	17	43.3
39	3	36	53.3
23	2	21	46.7
45	3	42	60
20	1	19	50
22	3	19	50
8	1	7	50

Overall wt cell number in *cxcr4a+/-; cxcr4b-/-* primordia	Wt leader cell number in *cxcr4a+/-; cxcr4b-/-* primordia	Wt follower cell number in *cxcr4a+/-; cxcr4b-/-* primordia	% completed migration
38	2	36	60
45	4	41	56.7
23	1	22	33.3
11	1	10	6.7
26	3	23	50
31	1	30	46.7
14	2	12	23.3
78	2	76	60
22	2	20	40
21	3	18	43.3
15	2	13	26.7
55	4	51	60
26	5	21	46.7
16	2	14	36.7
19	2	17	46.7
16	1	15	36.7

Figure S2. Wild-type cells in *cxcr4b* mutant primordia partly restore primordium migration, Related to Figure 2

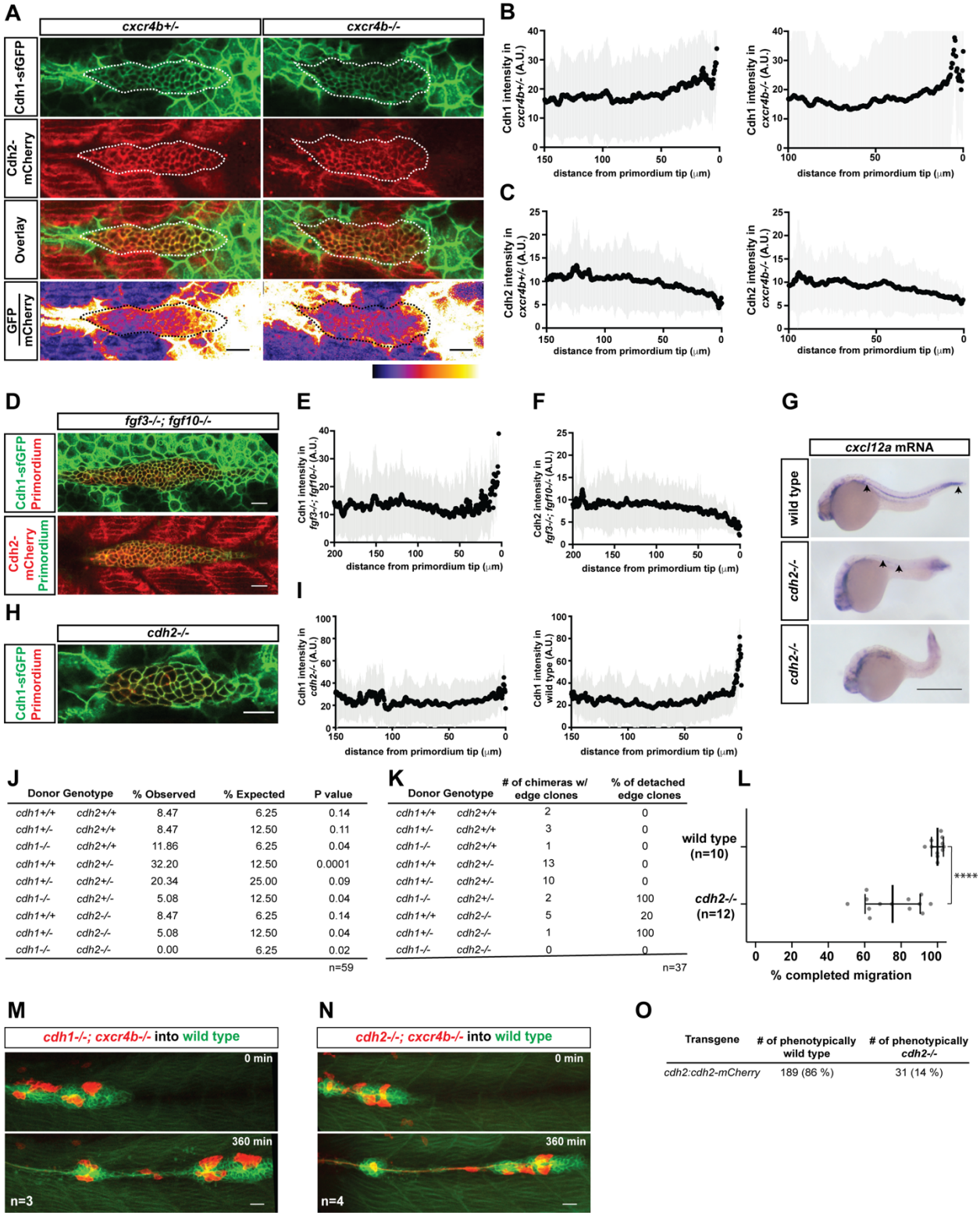
(A-C) Sum projections of 48 hpf embryos with chimeric primordia. Wild-type donor-derived cells in primordia labeled in red with *prim:lyn$_2$mCherry* and *cxcr4b*-/- host-derived cells labeled in green with *cldnB:lyn$_2$GFP* (left) and magnifications of chimeric primordia outlined by rectangles in A-C (A'-C'). The chimeric primordia are outlined by a dashed rectangle and asterisks mark the end of the yolk extension in A-C. Scale bars in A-C and A'-C' correspond to 100 μm and 20 μm, respectively.

(D) Graph of the migration distance of *cxcr4b* mutant primordia versus the total number of wild-type cells in the chimeric primordia (total number of wild-type cells include neuromast (nm) cells and primordium (prim) cells).

(E) Graph of the number of wild-type primordium leader cells versus migration distance of *cxcr4b* mutant chimeric primordia.

(F) Graph of the number of wild-type primordium follower cells versus migration distance of *cxcr4b* mutant chimeric primordia.

(G) Table summarizing the analysis of the migration defects of *cxcr4a*+/-*; cxcr4b*-/- and *cxcr4b*-/- primordia harboring different numbers of wild-type donor cells. The total number of wild-type donor cells in the host primordium and neuromasts, in the leader region only and the follower region only are indicated.



J

Donor Genotype		% Observed	% Expected	P value
cdh1+/+	*cdh2+/+*	8.47	6.25	0.14
cdh1+/-	*cdh2+/+*	8.47	12.50	0.11
cdh1-/-	*cdh2+/+*	11.86	6.25	0.04
cdh1+/+	*cdh2+/-*	32.20	12.50	0.0001
cdh1+/-	*cdh2+/-*	20.34	25.00	0.09
cdh1-/-	*cdh2+/-*	5.08	12.50	0.04
cdh1+/+	*cdh2-/-*	8.47	6.25	0.14
cdh1+/-	*cdh2-/-*	5.08	12.50	0.04
cdh1-/-	*cdh2-/-*	0.00	6.25	0.02

n=59

K

Donor Genotype		# of chimeras w/ edge clones	% of detached edge clones
cdh1+/+	*cdh2+/+*	2	0
cdh1+/-	*cdh2+/+*	3	0
cdh1-/-	*cdh2+/+*	1	0
cdh1+/+	*cdh2+/-*	13	0
cdh1+/-	*cdh2+/-*	10	0
cdh1-/-	*cdh2+/-*	2	100
cdh1+/+	*cdh2-/-*	5	20
cdh1+/-	*cdh2-/-*	1	100
cdh1-/-	*cdh2-/-*	0	0

n=37

O

Transgene	# of phenotypically wild type	# of phenotypically *cdh2-/-*
cdh2:cdh2-mCherry	189 (86 %)	31 (14 %)

Figure S3. Cadherin expression in wild-type, *fgf* mutant and *cdh2* mutant embryos and behavior of *cadherin*; *cxcr4b* double mutant clones in the primordium, Related to Figure 3 and Videos S1 and S2

(A) Single confocal section through primordium in live *cdh1:cdh1-sfGFP*; *cdh2:cdh2-mCherry* embryos heterozygous (left) and mutant for *cxcr4b* (right) at 36 hpf. Bottom panels show Cdh1-sfGFP-to-Cdh2-mCherry fluorescent intensity ratios as fire map.

(B) Quantification of Cdh1-sfGFP fluorescent intensity in embryos heterozygous (left, n=10) and mutant for *cxcr4b* (right, n=14) at 33-36 hpf. Fluorescent intensities were averaged across the front-to-back axis of the primordia. Mean and SD are shown.

(C) Quantification of Cdh2-mCherry fluorescent intensity in embryos heterozygous (left, n=12) and mutant for *cxcr4b* (right, n=8) at 33-36 hpf. Fluorescent intensities were averaged across the front-to-back axis of the primordia. Mean and SD are shown.

(D) Single confocal sections through primordium in live *fgf3*-/-; *fgf10a* -/- embryos transgenic for *prim:lyn$_2$-mCherry*; *cdh1:cdh1-sfGFP* (top) and *cldnB:lyn$_2$-GFP*; *cdh2:cdh2-mCherry* (bottom) at 36 hpf.

(E) Quantification of Cdh1-sfGFP fluorescent intensity in *fgf3*-/-; *fgf10a* -/- embryos at 36 hpf. Fluorescent intensities were averaged across the front-to-back axis of the primordia. Mean and SD are shown. n=3.

(F) Quantification of Cdh2-mCherry fluorescent intensity in *fgf3*-/-; *fgf10a* -/- embryos at 36 hpf. Fluorescent intensities were averaged across the front-to-back axis of the primordia. Mean and SD are shown. n=7.

(G) *In situ* hybridization against *cxcl12a* mRNA in wild-type (top) and *cdh2* mutant (middle and bottom) embryos at 25 hpf. Arrows point to the beginning and end of the *cxcl12a* stripe that guides the primordium. In *cdh2* mutant embryos the *cxcl12a* expression stripe is either dimmer and disrupted (middle) or completely absent (bottom). Scale bar corresponds to 0.5 mm.

(H) Single confocal section through primordium in live *cdh2*-/- embryos transgenic for *prim:lyn$_2$-mCherry*; *cdh1:cdh1-sfGFP* at 33 hpf.

(I) Quantification of Cdh1-sfGFP fluorescent intensity in *cdh2*-/- (left, n=5) and wild-type embryos (right, n=5) at 33 hpf. Fluorescent intensities were averaged across the front-to-back axis of the primordia. Mean and SD are shown. Note that fluorescent intensities are higher due to a microscope upgrade.

(J) Table summarizing the chimeric analysis of cadherin deficient donor cells in wild-type host primordia. Genotypes of cadherin clones in the primordia, observed and expected frequency of clones, p values and sample size are indicated.

(K) Table summarizing the chimeric analysis of cadherin deficient cells that are located at the edge of the primordium in wild-type primordia. Genotypes of cadherin clones, number of chimeras with edge clones of the indicated genotypes, the frequency of clones detaching from the migrating primordium and the sample size are indicated.

(L) Quantification of primordium migration in wild-type and *cdh2* mutant embryos at 48 hpf. Grey dots are individual data points. Sample size is as indicated. Vertical and horizontal black lines indicate mean and SD, respectively. **** = $p < 0.0001$ (Mann-Whitney test).

(M, N) Images of individual time points of sum projected time lapse videos of wild-type primordia (green) with clones of *cdh1*-/-; *cxcr4b*-/- (J) and *cdh2*-/-; *cxcr4b*-/- (K) primordium cells (red). The time lapse videos start at 28 hpf and stop at the indicated lapsed times. Scale bar is 20 μm.

(O) Table summarizing the outcomes of crosses between *cdh2:cdh2-mCherry*; *cdh2*-/+ and *cdh2*-/+ fish. The expected frequency for *cdh2* mutant embryos is 25 % if the *cdh2-mCherry* transgene does not restore Cdh2 function and 12.5 % if it restores Cdh2 function. Note that no phenotypically *cdh2* mutant embryos with red fluorescent eyes from the *cryaa:dsRed* BAC - transgene marker were found.

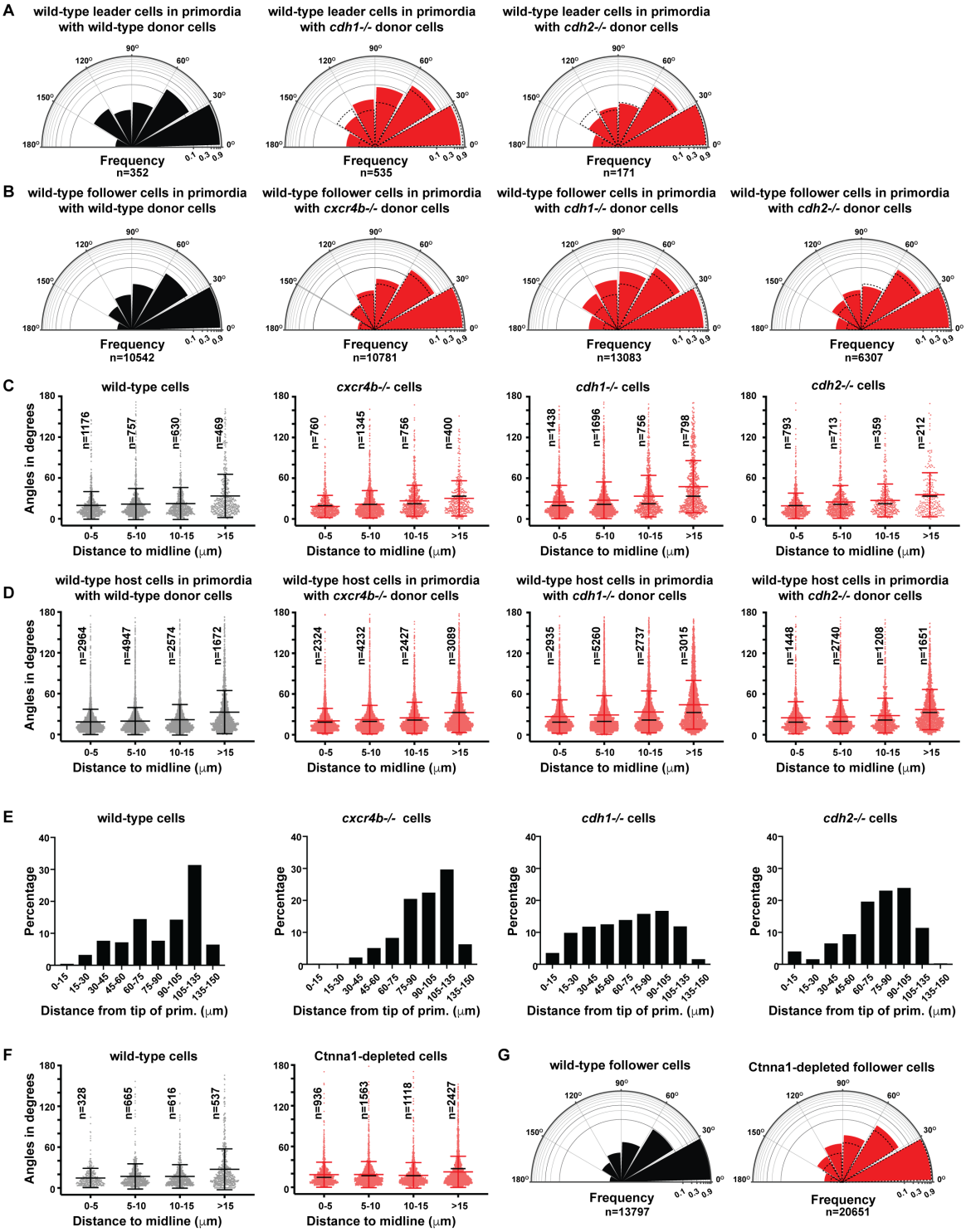

A
wild-type leader cells in primordia with wild-type donor cells
wild-type leader cells in primordia with cdh1-/- donor cells
wild-type leader cells in primordia with cdh2-/- donor cells
Frequency
n=352
n=535
n=171
B
wild-type follower cells in primordia with wild-type donor cells
wild-type follower cells in primordia with cxcr4b-/- donor cells
wild-type follower cells in primordia with cdh1-/- donor cells
wild-type follower cells in primordia with cdh2-/- donor cells
n=10542
n=10781
n=13083
n=6307
C
wild-type cells
cxcr4b-/- cells
cdh1-/- cells
cdh2-/- cells
Angles in degrees
n=1176
n=757
n=630
n=469
n=760
n=1345
n=756
n=400
n=1438
n=1696
n=756
n=798
n=793
n=713
n=359
n=212
0-5
5-10
10-15
>15
Distance to midline (μm)
D
wild-type host cells in primordia with wild-type donor cells
wild-type host cells in primordia with cxcr4b-/- donor cells
wild-type host cells in primordia with cdh1-/- donor cells
wild-type host cells in primordia with cdh2-/- donor cells
n=2964
n=4947
n=2574
n=1672
n=2324
n=4232
n=2427
n=3089
n=2935
n=5260
n=2737
n=3015
n=1448
n=2740
n=1208
n=1651
E
wild-type cells
cxcr4b-/- cells
cdh1-/- cells
cdh2-/- cells
Percentage
0-15
15-30
30-45
45-60
60-75
75-90
90-105
105-135
135-150
Distance from tip of prim. (μm)
F
wild-type cells
Ctnna1-depleted cells
n=328
n=665
n=616
n=537
n=936
n=1563
n=1118
n=2427
G
wild-type follower cells
Ctnna1-depleted follower cells
n=13797
n=20651

Figure S4. Directional angles and clone distribution for chimeric and Ctnna1-depleted primordia, Related to Figure 4 and Videos S3 and S4

(A) Semi-circular histogram plots of the directional angle frequencies (binned in 30 degree intervals) of wild-type leader cells in primordia with wild-type (left), *cdh1* mutant (middle) and *cdh2* mutant donor cells (right). Dashed lines indicate directional angle frequencies of the wild-type leader cells. The radial axis is plotted on a $\log_{10}$-scale. n indicates the total number of directional angles. The directional angle distributions for wild-type leader cells with wild-type donor cells and with *cdh1* mutant donor cells ($p = 0.0001$) are significantly different. The directional angle distributions for wild-type leader cells with wild-type donor cells and with *cdh2* mutant donor cells are not significantly different (Kolmogorov-Smirnov test).

(B) Semi-circular histogram plots of the directional angle frequencies (binned in 30 degree intervals) of wild-type follower cells in primordia with wild-type (far left), *cxcr4b* mutant (middle left), *cdh1* mutant (middle right) and *cdh2* mutant donor cells (far right). Dashed lines indicate directional angle frequencies of the wild-type follower cells. The radial axis is plotted on a $\log_{10}$-scale. n indicates the total number of directional angles. The directional angle distributions for wild-type leader cells with wild-type donor cells and with mutant donor cells are significantly different ($p < 0.0001$, Kolmogorov-Smirnov test).

(C) Distribution of directional angles as a distance from the midline of the primordium binned as indicated. Directional angles of wild-type (far left), *cxcr4b* mutant (middle left), *cdh1* mutant (middle right) and *cdh2* mutant cells (far right) in wild-type primordia are shown. Mean and standard deviation are shown. For the directional angles of the mutant cells, the mean of the corresponding directional angle of the wild-type cells is indicated in black. n indicates the number of directional angles for a given bin.

(D) Distribution of directional angles as a distance from the midline of the primordium binned as indicated. Directional angles of wild-type cells in primordia with wild-type (far left), *cxcr4b*

mutant (middle left), *cdh1* mutant (middle right) and *cdh2* mutant donor cells (far right) in wild-type primordia are shown. Mean and standard deviation are indicated. For the directional angles of the wild-type cells in primordia with mutant donor cells, the mean of the corresponding directional angle of the wild-type cells in primordia with wild-type donor cells is indicated in black. n indicates the number of directional angles for a given bin.

(E) Distribution of donor-derived wild-type (far left), *cxcr4b* mutant (middle left), *cdh1* mutant (middle right) and *cdh2* mutant cells (far right) in wild-type primordia along the front-back axis of the primordium.

(F) Distribution of directional angles of cells in wild-type control and Ctnna1-depleted primordia as a distance from the midline of the primordium binned as indicated. Mean and standard deviation are shown. For the directional angles of the Ctnna1-depleted cells the corresponding directional angle of the wild-type primordium cells is indicated in black. n indicates the number of directional angles for a given bin.

(G) Semi-circular histogram plots of the directional angle frequencies (binned in 30 degree intervals) of follower cells in wild-type (left) and Ctnna1-depleted primordia (right). Dashed lines indicate directional angle frequencies of the wild-type follower cells. The radial axis is plotted on a $\log_{10}$-scale. n indicates the total number of directional angles. The directional angle distributions for wild-type follower cells and Ctnna1-depleted follower cells are significantly different ($p < 0.0001$, Kolmogorov-Smirnov test).

cxcr4a genotyping primers: Outer PCR primers: 5'CCAACTTTGAGGTCCCGTGTGATG 3' 5' CTGTGGACACGGATGACATTCCTG 3' Inner PCR primers: 5'CCAACTTTGAGGTCCCGTGTGATG 3' 5' GAATGGCAGAGTGAGCAC 3'
cdh1 genotyping primers: Outer PCR primers: 5'CAATATAACAGGCTCTGGGCAGAT 3' 5' CAGACCAATTTTTACTGGTATCACC 3' Inner PCR primers: 5'TGTCCGTTATAGAGAGAAGC 3' 5' GTCAAGAATGCTTTGGATCG 3'
cdh2 genotyping primers: Outer PCR primers: 5' TGCAGTGTGTCTGTATGCGTAT 3' 5' AGACTGGATCTGAAACACACAC 3' Inner PCR primers: 5'AGTGATACTGTTTCCTCGGCAC 3' 5' TCTGACTGCACTTCACATGATG 3'
fgf3 genotyping primers: Outer PCR primers: 5' ATCCCGCCATGCCACAAT 3' 5' TCTCGTACCCCACATAAAACTGAC 3' Inner PCR primers: 5'CTGCTCTTGTTGTTACTGAGC 3' 5' CTCAAATATCAAACGGTTTACTCAC 3'
fgf10 genotyping primers: Outer PCR primers: 5' TGCATCACCCTTTCTCCCATCCAG 3' 5' TCGTCCTTGCTTTTGGTGCCATTG 3' Inner PCR primers: 5' GCTCTTCCCAGTTTTCCGAGCTCCAGGACAATGTGCA AATCG 3' 5' TCCGTTCTTATCGATCCTGAG 3'

Table S1. List of genotyping primer pairs, Related to STAR Methods.